PM&D/T&S B

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT 27 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: PP#8F3683/FAP8H5563. SENCOR® Herbicide (Metribuzin) In/On Alfalfa Seed, Alfalfa Chaff, Asparagus, Barley Forage, Barley Hay, Corn Silage, Corn Cannery Waste, Pea Straw, Wheat Hay, Tomato Processed Products and Sugarcane Molasses. EPA Reg. No's. 3125-314, 3125-325.

MRID #'s 400425-01, -02; 402555-01, -02; 402779-01, -02, -03, -05; 403717-01; 403676-01, -04, -05; 408027-01. Accession # 262890.

DEB #'s 4584, 4585.

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and

Toxicology Branch
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Mobay Corporation, Agricultural Chemicals Division is proposing tolerances for residues of metribuzin, 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5-(4H)-one and its triazinone metabolites on a number of raw agricultural commodities (racs) and processed products as follows:

Alfalfa, seed	0.1 ppm
Alfalfa, chaff	1.0 ppm
Asparagus	0.1 ppm
Barley, forage	2.0 ppm
Barley, hay	7.0 ppm
Corn, silage	0.1 ppm

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Corn, fresh, cannery waste	0.1 ppm
Pea, straw	4.0 ppm
Wheat, hay	7.0 ppm
Tomato, processed products	0.2 ppm
Sugarcane, molasses	2.0 ppm

This petition was submitted in response to the data requirements put forth in the Registration Standard for this chemical.

Tolerances for the combined residues of 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5-(4H)-one and its triazinone metabolites have been established under 40 CFR 180.332 for a number of racs, including, among others, asparagus (0.05 ppm); green alfalfa (2 ppm); alfalfa, hay (7 ppm); barley, grain (0.75 ppm); barley, straw (1 ppm); corn, fresh (inc. seed K + CWHR) (0.05 ppm); corn, fodder and forage (0.1 ppm); corn, grain (inc. popcorn) (0.05 ppm); peas (0.1 ppm); peas (dried) (0.05 ppm); peas, forage (0.5 ppm); peas, vine hay (0.05 ppm), sugarcane (0.1 ppm); tomatoes (0.1 ppm); wheat, forage (2 ppm); wheat grain (0.75 ppm); wheat, straw (1 ppm). Tolerances on animal products in §180.332 include eggs (0.01 ppm); milk (0.05 ppm) and the fat, meat byproducts and meat of cattle, goats, hogs, horses, poultry, and sheep (0.7 ppm).

Food additive tolerances have been established under 40 CFR 185.250 for barley; milled fractions (except flour) (3 ppm); potatoes, processed (inc. potato chips) (3 ppm); sugarcane molasses (2 ppm); and wheat, milled fractions (except flour) (3 ppm).

Feed additive tolerances have been established under 40 CFR 186.250 for barley, milled fractions (except flour) (3 ppm); potato waste, processed (dried) (3 ppm); sugarcane bagasse (0.5 ppm); sugarcane molasses (0.3 ppm); tomato pomace (dried) (2 ppm); and wheat, milled fractions (except flour) (3 ppm).

Conclusions

1a. The directions for use of metribuzin on alfalfa and field corn contain no seasonal maxima. The registrant should submit a revised Section B proposing maximum seasonal use levels on these commodities. Also, the proposed seed use for alfalfa with PHI of 180 days should be included in the use label.

1b. No PHI's appear in the use label for winter wheat. The registrant should submit a revised Section B specifying an appropriate PHI.

2. The nature of the residue in plants is not adequately understood. The following conclusions apply to plant metabolism:

   a. In the soybean metabolism study, "Metabolite 15" accounted for 17.0% of the total recovered radioactive residue (TRR) in soybean plant tissue. The registrant should better characterize this metabolite using suitable physical methods (NMR, mass spec., etc.).

   b. Similarly, in the soybean study, the registrant should better characterize "Metabolite 11", found at 10.7% of the TRR in soybean seeds.

   c. The nature of the residue in wheat forage and wheat straw is adequately understood. The residue to be regulated consists of metribuzin and its triazinone metabolites. However, in grain almost 50% of the TRR consisted of water-soluble residue that apparently was not characterized. The registrant should characterize the metabolites in the aqueous phase using TLC or HPLC. Any individual metabolite present at over 10% of the TRR must be further characterized using suitable physical methods.

3. The nature of the residue in ruminants is not adequately understood. The nature of the residue in poultry is understood pending response to certain questions. The following conclusions apply to animal metabolism:

   a. Percent recoveries of radioactivity -- the total recovered by the various extraction procedures divided by the radioactivity determined by combustion and liquid scintillation counting times 100 -- should be reported for both the ruminant and the poultry metabolism studies. Such recoveries were reported in the two plant metabolism studies.

   b. In the goat metabolism study "Unknown #9" was present in liver at 17.6% of the TRR. Further characterization is necessary. Data showing the identity of 2N hydrolysis products from this individual metabolite may be acceptable.

   c. "Unknowns #8a and #8b" were present in kidney at 19.7% and 18.1% of the TRR, respectively. These same unknowns were present in milk at 43.0% and 40.9% of the TRR, respectively. Further characterization is necessary. Specific acid hydrolysis data (including time and temperature) should be submitted. Attempts should be made to identify the unknowns by physical

methods such as NMR or mass spectrometry. Absolute identification may not be possible, but we would like to know with more certainty the identity of the metribuzin-related portions of the metabolites.

d. The nature of the residue in muscle and fat of ruminants is understood, pending satisfactory response to Conclusions 3a and 3e. The principal constituents of the residue are parent, triazinone metabolites and butylthion. This latter metabolite is also found in liver and in kidney.

Butylthion has been found to be a residue in ruminants. The metabolite has not been isolated from plants. However, due to very low anticipated residues (<0.01 ppm) in meat, DEB does not consider it necessary that this metabolite be regulated unless TOX has concern.

e. Representative copies of TLC's used in identifying the ruminant and poultry metabolites should be submitted.

f. The nature of the residue in poultry and eggs is adequately understood pending satisfactory response to Conclusions 3a and 3e. The residue to be regulated is metribuzin and its triazinone metabolites.

4a. Analytical methods available for enforcement appear in PAM II. However, it is not clear that the extraction procedures of either the method described in Mobay Report No. 40901, for plant analysis, or Report No. 42257, for animal tissue analysis, will release triazinone metabolites to the same extent as the extraction procedures in the metabolism studies. The registrant should analyze radiolabeled-treated wheat straw and soybean tissue using method 40901 and compare results to those from the respective metabolism studies. Similarly, goat kidney from radiolabeled-treated metribuzin should be analyzed by the residue analytical method 42257 and the results compared to those from the metabolism study.

4b. The residue analytical method for plants was only generally described in the petition. The petitioner should submit all modifications to the original method for each of the racs analyzed in this petition.

4c. Metribuzin was not recovered by FDA multiresidue Method #2 (Protocol B) and was completely recovered by Method #4 (Protocol D). The pesticide should be tested through the remaining three protocols of PAM I. The "Decision Tree for MRM Testing" should be used as a guide.

5a. Assuming that residues are stable under frozen storage (see Conclusion 8a), the wheat forage data support the current tolerance of 2.0 ppm. The wheat hay data support the proposed tolerance of 7.0 ppm.

5b. The wheat processing study requested in the Registration Standard is being redone because field trial residues were below the detection limit of the method (0.05 ppm).

6a. Storage stability data are adequate for alfalfa.

6b. Mobay has proposed a tolerance of 0.1 ppm for seed alfalfa and 1.0 ppm for alfalfa chaff as a result of 3 field trials. The tolerances are appropriate, but we note that we do not normally set tolerances on alfalfa chaff and do not recommend that we do so for this petition. As noted in Conclusion 1, the registrant should submit a revised Section B which includes alfalfa seed with the accompanying PHI of 180 days.

7a. Storage stability data on frozen green peas are adequate to support dry pea frozen storage.

7b. The residue data support the current tolerance of 0.05 ppm for dried peas.

7c. We consider the tolerance of 4.0 ppm for "pea vine hay" (pea straw) to be appropriate.

8a. Storage stability data are lacking for corn. Mobay must produce data showing that metribuzin residues are stable in corn grain and fodder held in frozen storage for up to 253 days.

8b. Assuming storage stability, the data support current tolerances of 0.05 ppm for grain and 0.1 ppm for forage and fodder.

8c. The requested corn processing study was not completed because residues were near the detection level. However, because detectable residues were obtained and further concentration in processed products is possible, the processing study should be completed.

8d. The tolerance for corn forage includes that for corn silage. No additional silage data are necessary, nor is a separate tolerance for silage necessary.

8e. As requested by the Registration Standard, Mobay has proposed a feed additive tolerance of 0.1 ppm for fresh

corn cannery waste.

9. On the recommendation of the Registration Standard, Mobay has proposed a tolerance increase from 0.05 ppm to 0.1 ppm for metribuzin residues on asparagus.

   The field trial data submitted in this petition for asparagus, while consistent with the tolerance, are not supported by storage stability data. This conclusion does not affect the proposed tolerance, which is appropriate.

10. On the recommendation of the Registration Standard, Mobay has proposed a food additive tolerance of 0.2 ppm for "tomatoes, processed products".

11a. On the recommendation of the Registration Standard, Mobay has included a grazing restriction on its use label for sugarcane grown in Hawaii.

11b. A previously submitted sugarcane processing study was rejected by the Registration Standard because parent and two of three metabolites were undetectable. On reviewing the study we agree with the registrant that the study was adequate. Our guidelines state that residues in the sample to be processed must be at or near the tolerance but make no mention of metabolite distribution in the raw commodity. Criteria in the guidelines were met by Mobay's sugarcane processing study.

12. Residue levels in meat, milk, poultry and eggs are not directly affected by this petition. However, see our concluding note, below.

13. An International Residue Limit Status sheet is attached to this review. The U.S. tolerance expression is not in agreement with its Canadian counterpart because TOX has deemed it necessary that the triazinone metabolites be regulated. Also, the numerical tolerance for barley grain -- 0.75 ppm -- is higher than the Canadian 0.1 ppm tolerance.

Final Note:

Conclusions concerning residues on plants and animals are provisional pending response to Conclusion #4a concerning the adequacy of the analytical methods and Conclusion #3d concerning the animal metabolite butylthion.

Recommendations

DEB cannot recommend for the tolerances of this petition due to Conclusions 1a and 1b (label); 2a,b,c (plant metabolism); 3a,b,c,e (animal metabolism); 4a,b,c (analytical methods); 5b (wheat processing study); 6b (alfalfa seed use label); 8a,c (corn storage stability, processing study).

TOX should comment on the toxicity of butylthion, a ruminant metabolite not found in plants (see Conclusion 3d).

NOTE TO PM: The feed additive tolerance of 0.3 ppm for metribuzin residues in sugarcane molasses, as listed in 21 CFR 561.41 and 40 CFR 186.250 is in error. The correct tolerance, published in the Federal Register in 1978, should be 2.0 ppm.

Detailed Considerations

Manufacture and Formulation

The manufacturing equations and certification of limits are given in Mobay Chemical Co.'s response to the Registration Standard, as reviewed by G. Makhijani in his memo of 6/26/86. The active ingredient (ai) constitutes a minimum of 90% of technical metribuzin. Results of anlysis of five batches of metribuzin are also given in the 6/26/86 memo. No major residue problem is expected from the impurities.

Metribuzin is currently formulated as SENCOR® 4 Flowable Herbicide, EPA Reg. No. 3125-314, which contains 41% ai (4 lbs ai/gal), and SENCOR DF® 75% Dry Flowable Herbicide, EPA Reg. No. 3125-325, which contains 75% ai.

Proposed Use

Labeling for the two SENCOR® products on the crops named in this petition has already been accepted for registration. Use directions for the two formulations are equivalent based on active ingredient. Application can be made by ground or air.

Alfalfa: Recommended broadcast application levels vary to 1 lb ai/A depending on state and soil type.

Do not graze or harvest within 28 days after application.

Treat only dormant established alfalfa (with some exceptions specified on the label). Do not apply after growth begins in the spring or before growth ceases in the fall.

Comment -- The label should contain a seasonal maximum use level.

Asparagus: Recommended broadcast application levels are 1-2 lbs ai/A for preemergence application, 1/2-1 lb/A pre-emergence plus 1-1.5 lbs ai/A postharvest for split applications.

The total amount of SENCOR® applied in one crop season may not exceed 2 lbs ai/A.

Do not apply post harvest applications until after The last harvest of spears.

Field Corn (IA, MO, NE and SD only): SENCOR® is applied preemergence at 0.25 lb ai/A in tank mixes with Lasso plus atrazine, Lasso plus Bladex, Dual plus atrazine, or Dual plus Bladex.

Comment -- SENCOR 4 F and SENCOR DF are not registered for use on sweet corn.

Peas (ID, OR, WA and MT): Recommended preemergence application rates are 1/4 - 3/8 lb ai/A. For postemergence application, use 1/8 - 3/16 lbs ai/A on spring peas, 3/16 - 1/4 lb ai/A on winter peas.

One postemergence application may be made per season.

Do not apply within 50 days of harvest of peas.

Do not graze or feed treated vines to livestock within 40 days after application.

Sugarcane:

For HI, apply 4-6 lbs ai/A preemergence or early postemergence or 2-4 lbs ai/A postemergence (2.5-5 lbs./A spot treatment). Do not apply more than 8 lbs ai/A/ crop cycle. Do not apply within 17 months of harvest. Do not use treated foliage for feed or forage.

For LA, TX, apply 1.5-3 lbs ai/A broadcast. Apply during the Fall after planting or to the stubble after harvest. Make a second application early in the Spring. For band application, apply 0.75-1.5 lb ai/A in bands two times -- as in broadcast application.

Do not apply within 60 days of harvest.

For FL, ground-apply 1-2 lbs ai/A postmergence. Do not use more than 2 lbs ai/A/season. Air-apply 1-1.5 lbs ai/A.

Do not apply within 60 days of harvest.

For all the above states: Do not use treated crop for feed or forage.

Tomatoes:

Apply 0.25-0.375 lb ai/A postemergence broadcast spray or 0.25-1 lb ai/A postemergence directed spray. Do not apply more than 1 lb ai/A/season. Allow at least 14 days between applications. Do not apply within 7 days of harvest.

Barley (ID, OR, MO, UT, WA):

Apply 0.25-0.50 lb ai/A depending on soil type and percent organic matter. Do not graze or harvest treated barley for feed before crop maturity. Apply only once per crop season.

Winter Wheat:

ID, OR, MT,, UT, WA. Apply 0.375-0.5 lb ai/A depending on soil type and percent organic matter. A single postemergence application is recommended.

OK, TX. Apply 0.25-0.75 lb ai/A postemergence broadcast, depending on soil type and percent organic matter, in Fall or Spring

AR, LA, MS, TE. Apply 0.25-0.50 lb ai/A postemergence broadcast.

Additional instructions apply to tank mixes with other pesticides and to crop rotations.

Comment -- The label should specify post harvest intervals (PHI's).

Nature of the Residue

Plants.

Radiolabeled studies using ^{14}C metribuzin were previously submitted for soybeans, potatoes, sugarcane, tomatoes and alfalfa. Reviewers have concluded that the nature of the residue in plants was adequately understood. (See, for example, PP#4E3112, L. Kutney, memo of 9/4/84.) Nevertheless, the Registration Standard concluded that the available data were inadequate because

large portions of the ^{14}C-residues recovered from

alfalfa (ca. 66%), potatoes (34%), soybeans (40%) and sugarcane (93%) were not identified....the only submitted study in which metabolism following post-emergence foliar treatment was studied was conducted on alfalfa; however, as stated above, 66% of the recovered residues was not identified.

A total of twelve radiolabeled studies were reviewed in the Registration Standard. Only in one potato study was sufficient characterization achieved. In that study white seed potato seedlings were grown in planting boxes filled with soil treated with radiolabeled metribuzin applied at 2 lb ai/A [3-^{14}C-label]. Immature tubers were harvested 65 days after treatment and after extraction and various hydrolysis steps, the following compounds were identified and quantitated: DK (29.6% of total ^{14}C activity), parent (21.3%), DADK (8.7%) and DA (6.8%) for a total of 66.4% of the total activity. (See Figure 1 for structures of metabolites.)

Two studies on alfalfa were the only ones in which metribuzin was applied to the upper parts of the plants. In one study, transplanted plants were sprayed once with [^{14}C]metribuzin at 0.5-1 lb ai/A and were harvested up to 250 days after treatment. Total ^{14}C residues in samples from the 41 day sampling interval were 0.37 ppm (as parent). By a reflux extraction procedure, 36.1% of the ^{14}C residue from a sample treated with metribuzin labeled in the 3C position and harvested at 49 days was found to be organosoluble, 55.1% was water soluble and 8.8% was insoluble. TLC of the organosoluble layer showed the following metabolites (as percent of the total radioactive residue): parent, 4.7%; DADK, 28.6%; DK, 0.5%; DA, 0.5%. In other words, only 34.3% of the total radiocarbon residue was characterized. (Results using 5C-labeled metribuzin were comparable.) [These figures were obtained from Mobay's Report 40853, submitted in PP#5F1628.] Attempts to release possible conjugated metabolites in the aqueous phase by using 8 different enzymes met with no success, and it is possible that the metabolites were irreversibly bound to plant peptides.

From the potato, alfalfa and the other studies, we conclude that metabolism of metribuzin in plants occurs -- at least in part -- via deamination and/or dethiomethylation to yield triazinone moieties and their conjugates.

The Registration Standard required the following two studies:

1. Data reflecting the distribution and metabolism of ring-labeled [^{14}C]metribuzin in mature soybeans (foliage and beans) following preemergent soil application at 0.5 lb ai/A. Analysis should include hydrolysis and reextraction of plant residues and

aqueous fractions to determine conjugated ^{14}C-residues of metribuzin.

2. Data reflecting the distribution and metabolism of ring-labeled [^{14}C]metribuzin in mature wheat (foliage and grain) following postemergence broadcast application at 0.75 lb ai/A. Analysis should include hydrolysis and reextraction of plant residues and aqueous fractions to determine conjugated ^{14}C-residues of metribuzin.

In response, Mobay Corporation has submitted two plant metabolism studies in response to the Registration Standard:

1. "Metabolism of ^{14}C-®SENCOR in Soybeans," M.F. Lenz, G.D.Parker and J.S. Hundal, 6/30/87, Laboratory Project ID # 94593 (MRID # 402555-02).

2. "Metabolism of ®SENCOR in Wheat," M.J. Schocken, I.M.R. Philippson and C.L. Burge, 6/23/87, Laboratory Project ID # 94592 (MRID # 402555-01).

Soybeans

A SENCOR spray solution was prepared by dissolving 33.75 mg ^{14}C-SENCOR, labeled in the carbonyl position and having specific activity of 20.8 mCi/mmol, and 48.57 mg SENCOR 4 formulation blank in 125 mL water. The purity of the spray solution was determined to be 97.3% by TLC. The solution was applied to Kansas sandy loam placed in five plastic 2.3 ft^2 tubs at a rate equivalent to 0.3 lbs ai/A. A sixth tub containing the same soil was not treated. Prior to treatment, but on the same day, 250 soybean seeds were planted in the soil.

A total radioactive residue of 0.32 ppm (metribuzin equivalents) was found in the 0-day soil and 0.15 ppm in the harvest day soil (114 days post treatment). Analysis of the 0-day soil showed that 87.0% of the recovered radioactivity was organosoluble. This fraction consisted chiefly of parent (69.6%) and a metabolite which degraded to parent after the compound was isolated from TLC analysis (14.0%). Characterization of metabolites in the harvest day soil was not reported.

Soybeans were harvested at maturity (114 days). Stalks, leaves, stems and bean pods ("plant tissue") were combined and ground in liquid nitrogen. Soybean seeds were ground separately. Aliquots of these solids were analyzed by oxidation to $^{14}CO_2$ and liquid scintillation counting (LSC). In this way the total radioactive residue in the harvest soybean plants was determined to be 12.1 ppm (metribuzin equivalents). The residue in mature soybean seeds was determined to be 0.48 ppm.

The extraction scheme for soybean tissue is given in Figure 2. 99.4% of the total radioactivity (as determined by LSC) could be recovered following this scheme but, as noted below, 13.6% of the determined residue remained unextractable ("bound").

Plant tissue was extracted with methanol/water (4/1) and filtered. The filtrate was evaporated to water and then partitioned with ethyl ether. The remaining solids were refluxed with 1N HCl, filtered, and the filtrate extracted with ethyl ether. The solids were then refluxed with 6N HCl, filtered, and the filtrate partitioned with ethyl ether. The organic and aqueous phases (refer to Figure 2) were analyzed by TLC and HPLC. Over 73% of the total recovered radioactivity (TRR) appeared in the initial organic and aqueous layers (Organic-1B, Aqueous-2B in Figure 2). Water soluble radioactivity (Aqueous-2B) comprised 44.2% of the TRR.

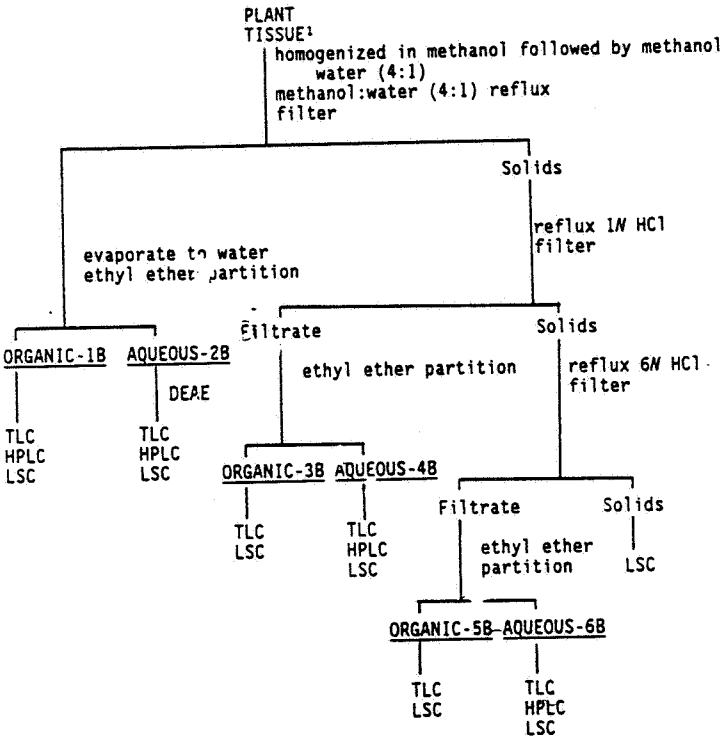


Figure 2. Extraction scheme used for the isolation of ^{14}C-SENCOR and its degradation products in soybean plants. Abbreviations: TLC - Thin-layer chromatography, LSC - Liquid scintillation counting, HPLC - High performance liquid chromatography.

[1]Plant tissue consists of stems, leaves, and pods combined for analysis.

Seven metabolites were identified in the organosoluble fraction (Organic-1B). DADK was found at 19.2% of the TRR. Minor residues of metribuzin, DK, hydroxy-t-butyl-DADK, hydroxy-t-butyl-metribuzin and 3-amino-DA accounted together for only 3.6% of the TRR. (Reproductions of the TLC plate and ^{14}C-TLC scan are given in Figure 6 of the report. The ^{14}C-HPLC chromatogram is given in Figure 7.)

The aqueous fraction (Aqueous-2B) was purified on a diethylaminoethyl column and components separated using HPLC and TLC. Aliquots of each of the major radioactive metabolites isolated in this way were individually incubated with α-D-glucoside at 37°C in a phosphate buffer (pH 7.0) for 16 h. After incubation all samples were partitioned with ether. Both phases were radioassayed by LSC, and the organic phase was analyzed by TLC.

Five "major" metabolites were isolated in Aqueous-2B by HPLC and purified by TLC and HPLC. An additional 15 metabolites appeared as discrete peaks in the HPLC chromatogram corresponding to concentrations not exceeding 1% of the total recovered radioactivity. Aliquots of the five major metabolites were individually incubated with α-D-glucoside at 37°C in a phosphite buffer (pH 7.0) for 16 h. Under these conditions 2 of the 5 components released DADK. The metabolite present at the highest concentration of the 5, "Metabolite 15", comprising 17.0% of the TRR, was not identified. Concentrations of the remaining "major" metabolites did not exceed 5% of the TRR.

Acid hydrolysis of the residue remaining after the original methanol/water extraction released additional DADK at 3.4% of the TRR. DK and hydroxy-t-butyl-DADK were found at concentrations lower than 0.5% of the TRR.

In all, 35.8% of the total recovered residue was identified, of which DADK and its conjugates accounted for 31.8%. The registrant should better characterize "Metabolite 15", which accounted for 17.0% of the TRR. Based on total radiocarbon residue, the metabolite was present at 2.1 ppm in metribuzin equivalents. The remainder of the residue was comprised of numerous metabolites, no one of which exceeded 5% of the TRR. Further characterization of these metabolites is not necessary.

The extraction procedure for soybean seeds was identical to that given for soybean plant tissue except that the solids remaining after 1N HCl reflux were not further refluxed in 6N HCl. Of the initial radioactive residue, 95.8% could be recovered following the procedure. Of that percentage, 7.0% could not be released by solvent extraction or acid hydrolysis.

A total of 45.2% of the TRR could be identified, of which 44.5% was DADK, free or conjugated. Hydroxy-t-butyl-DADK

constituted the remainder of the identified residue. Eleven metabolites were isolated by HPLC of the aqueous fraction (Aqueous-2B). Only one was present in amounts greater than 5% of the TRR -- the percentage of Metabolite 11 was 10.7%, or 0.05 ppm in metribuzin equivalents, based on the total radiocarbon residue of 0.48 ppm. The ^{14}C-HPLC chromatogram of Aqueous-2B shows a well-resolved peak for Metabolite 11. The petitioner should attempt to identify this metabolite using appropriate methods, such as NMR or mass spectrometry. We note that from comparison of chromatograms, Metabolite 11 is not "Metabolite 15", the principal unidentified metabolite from plant tissue.

Four soybean studies were reviewed in the Registration Standard. In the one study in which an attempt was made to characterize metabolites after preemergent application (PP#2F1274, Report No. 29800) parent and DADK were found. However, only a small fraction (<10%) of the organosoluble fraction from plants harvested after 7 weeks was identified, and although aqueous fractions were submitted to acid and enzymatic hydrolysis, no attempt was made to provide total metabolite concentrations (i.e., concentrations from organic + aqueous + solids) at a given plant harvest time. The study is not useful.

General Comment

The 1987 study indicates that the main metabolic pathway of metribuzin in soybeans involves oxidation of the methylthio group, deamination, and conjugation with glucose. Numerous minor metabolites are produced. Once a satisfactory attempt has been made to identify Metabolites 11 and 15, we can consider this to be an acceptable metabolism study.

Wheat

The metribuzin used in this study was prepared with a 1:1:1 ratio of ^{12}C:^{13}C:^{14}C in the 5 position. The specific activity was 20.8 mCi/mmole. A solution prepared by adding 39.6 mg to 220 mL water was sprayed onto 2.3 ft^2 trays containing wheat grown to the 3-tiller stage. (The number of treated plants was not reported.) Each tray received 3.6 mg metribuzin, the maximum tolerable dose. The application rate is equivalent to 0.15 lb ai/A. Forage samples were collected immediately after application and 7 days after application. Final harvest -- wheat straw and grain -- occurred 33 days after application. Zero and 7 day forage samples were ground in liquid nitrogen and radioactivity determined by combustion to $^{14}CO_2$ and LSC analysis. With the 33 day harvest, the wheat influorescences were cut with scissors and the straw was cut to within 1-2 inches of the ground. The influorescences were separated into grain and chaff and the chaff discarded. The grain and straw were ground in liquid nitrogen and analyzed by LSC. Total radioactive residues in metribuzen equivalents are given in Table 1.

Table 1

Plant Part	PHI(days)	Metribuzin Equivalents(ppm)
Forage	0	5.4
Forage	7	1.2
Straw	33	5.5
Grain	33	0.2

Analysis of Day 0 Forage. The ground forage sample was extracted twice for 15 minutes with methanol:water (80:20). The extracted ground plant material was filtered, and the filtrate taken to dryness. The residue was redissolved in a few mL of 80% MeOH and an aliquot analyzed by TLC and autoradiography.

At the time of extraction, several hours after application, 16% of the radiocarbon residue was already unextractable. TLC of the methanol extract showed one band having identical R_f to the metribuzin reference standard. No breakdown products were detected by visual inspection of the autoradiogram.

Analysis of Day 7 Forage. The extraction scheme for Day 7 forage is given in Figure 3.

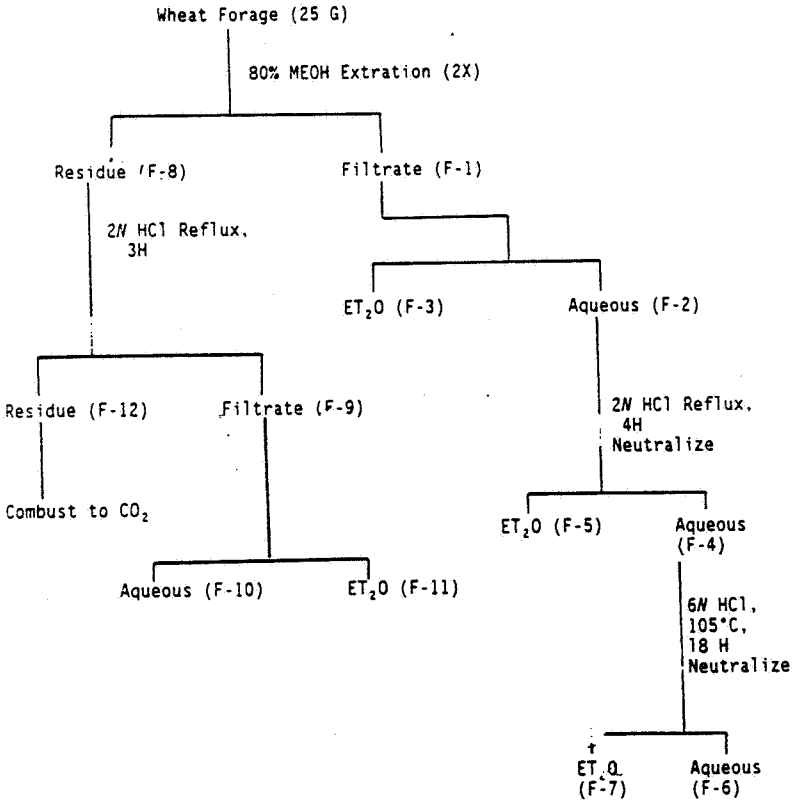


Figure 3. Scheme describing the steps involved in the extraction and isolation of SENCOR metabolites from 7-day wheat forage.

Ground plant material (25g) was extracted as described for the day-0 forage. The extract was roto-evaporated to water. A portion of this concentrate (5mL) was further concentrated for TLC analysis, while the remainder (77mL) was extracted with diethyl ether (Et_2O). The ether was evaporated, the residue dissolved in 1-2 mL MeOH, and the solution analyzed by TLC and HPLC. The residual aqueous phase was acidified to ≈2N with concentrated HCl and heated in a boiling water bath for 4 hours (to hydrolyze water-soluble conjugates, such as N- and O-glycosides). The solution was then neutralized (NaOH), extracted with Et_2O, as before, and analyzed by HPLC and TLC. The water in the remaining aqueous phase was removed by rotary evaporation and the residue redissolved in 6N HCL and heated at 105°C for 18 h in a 10-mL Reactivial (to hydrolyze water soluble peptide conjugates which would not be expected to hydrolyze in 2N HCl). The solution was cooled, neutralized and extracted with ether. The ether phase was analyzed by TLC and HPLC, as above. The unextractable radioactivity was determined in the usual way. Results will be discussed below (Table 2).

Analysis of Wheat Straw (Day 33). Wheat straw was extracted with 80% MeOH, as above. The extract was treated in identical manner to that from day 7 forage. Since a larger percentage of the radioactivity was not extracted by this procedure, the remaining residue was subjected to a more complicated extraction process. The residue (F8 in Figure 3) was refluxed in 2N HCl for 4h. and the neutralized filtrate extracted with ether and analyzed as before, but the remaining aqueous layer was then acidified to 6N and heated in a reactivial at 103°C for 18h. The neutralized solution was extracted with ether and analyzed by TLC. The previously MeOH-extracted and acid-refluxed residue (F12) was refluxed for 4h. in 1N NaOH, then filtered. The solid residue was analyzed by combustion to $^{14}CO_2$. the filtrate was neutralized, extracted with ether and analyzed by TLC and HPLC, as above. Results are discussed below.

Analysis of Wheat Grain (Day 33). The extraction procedure for wheat grain differed significantly from the previous extractions and was more complicated (Figure 4).

Wheat grain was extracted with n-hexane. The extract was concentrated and passed through a silica gel Sep-Pak cartridge. The cartridge was eluted with MeOH and the eluate analyzed by TLC.

The plant material remaining after the hexane extraction was then extracted with 80% MeOH. Upon concentrating the MeOH filtrate by roto-evaporation to water solution, a white precipitate formed which could be dissolved by successive treatments with water, dioxane and DMSO. The concentrated aqueous layer from the MeOH extract was extracted with ethyl acetate (EtOAc) and aliquots analyzed by HPLC and TLC. The

aqueous layer remaining after EtOAc extraction was reextracted with EtOAc for 14h. using a liquid-liquid continuous extractor. The EtOAc phase was analyzed by TLC. The aqueous phase was adjusted to 2N with concentrated HCl and refluxed. The resulting solution was extracted with EtOAc and analyzed by TLC.

The solid residue remaining after successive hexane and 80% MeOH extractions was refluxed in 2N HCl for 4h. The neutralized filtrate was extracted with EtOAc, and the residue from the acid reflux was further refluxed in 6N HCl for 8h. The neutralized filtrate was extracted with EtOAc.

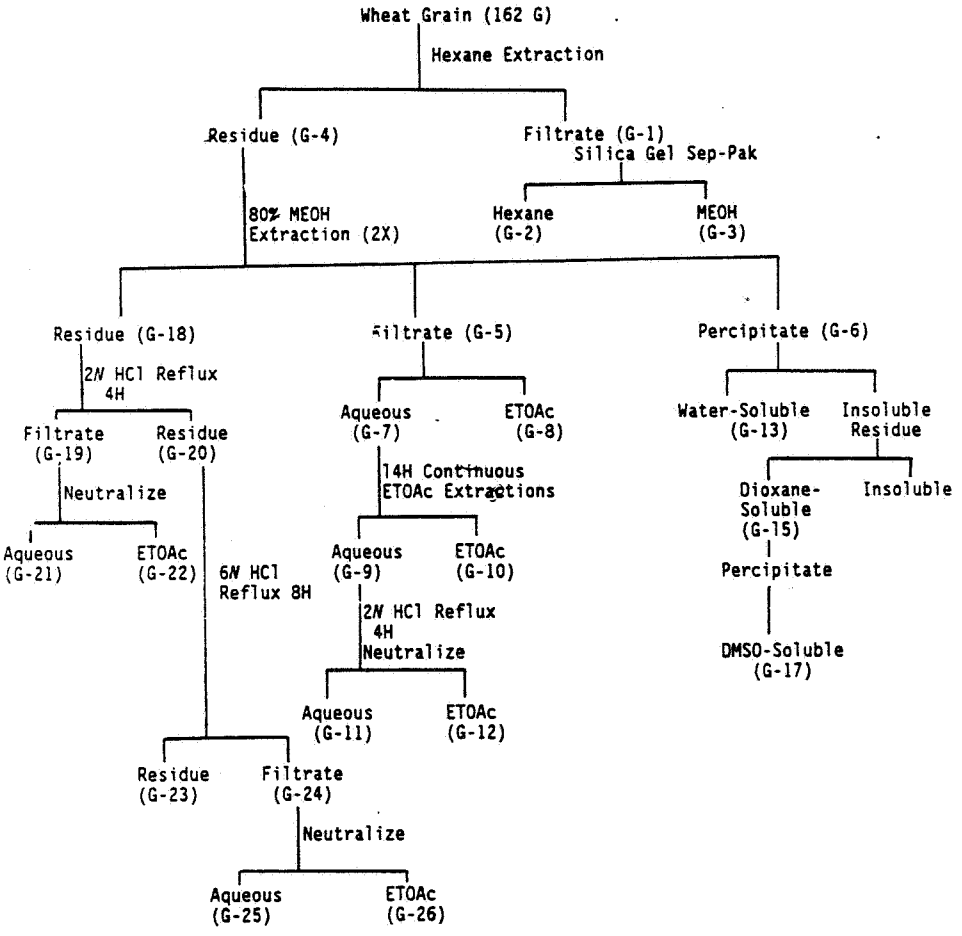


Figure 4. Scheme describing the steps involved in the extraction and isolation of SENCOR metabolites from 33-day wheat grain.

Results and Discussion

Results are summarized in Tables 2a, 2b and 2c (similar to Table IX in the petition).

Table 2a*

7-Day Wheat Forage

Percent of TRR		
Identified Metabolites		
Metribuzin	42.8	
DADK	6.8	
DK	7.5	
Total	57.1	(Equivalent to 0.68 ppm in metribuzin equivalents based on Table 1)
Unidentified Metabolites		
80% MeOH-extractable		
Organo-soluble	8.2	(diffuse TLC bands)
Water-soluble	14.4	(12.3% remained water soluble after heating with 6N HCl at 105°C for 18h.)
"Bound Residues" -- residues not extracted by 80% MeOH but extractable with 2N HCl.		
Water soluble	7.5	
"Metabolite #2"	1.1	
Unextractable -- radioactivity not extracted with 80% MeOH and 2N HCl.	11.7	

* Percent recovery -- the sum of radioactivity contained in all terminal fractions divided by the total plant-associated radioactivity (as determined by combustion to $^{14}CO_2$) x 100 -- was 76.2%.

Table 2b*

33-Day Straw

Percent of TRR		
Identified Metabolites		
Metribuzin	3.9	
DADK	11.2	
DK	3.5	
DA	0.7	
3-Amino-DA	4.3	
t-BuOH-DADK	0.6	
Total	24.2	(equivalent to 1.3 ppm metribuzin equivalents based on Table 1)
Unidentified Metabolites		
80% MeOH-extractable		
Organo-soluble	5.1	(diffuse TLC bands 1.6%, origin 3.5%)
Water-soluble	22.2	(From organosoluble fractions arising from 2N and 6N hydrolysis, 5 unidentified metabolites, all <1% of the TRR, were isolated. 17.1% remained water soluble after heating in 6N HCl at 105°C for 18h.)
"Bound Residues"	45.5	(Includes residues solubilized by 2N HCl reflux and residues solubilized

by the 1N NaOH reflux. 15.6% of the TRR remained water soluble after heating in 6N HCl at 105°C for 18h. 25.6% of the TRR remained water soluble after 1N NaOH 4h. reflux.)

Unextractable	3.0	(Radioactivity not extractable with 80% MeOH, 2N HCl and 1N NaOH.)

* Percent recovery was 79.4%.

Table 2c*

Wheat Grain

Percent of TRR		
Identified Metabolites		
Metribuzin	1.8	
DADK	3.9	
DK	1.1	
DA	0.7	
t-BuOH-DADK	1.3	
t-BuOH-DA	0.5	
Total	9.3	(equivalent to 0.2 ppm in metribuzin equivalents based on Table 1)
Unidentified Metabolites		
80% MeOH-extractable		
Organosoluble	7.1	(Includes 3 unknown metabolites at concentrations 0.4-1.4% of the TRR. 3.0% remained at the orgin of the TLC plate.)
Water soluble	24.3	(20.2% remained water soluble after 2N HCl reflux (G-11).)
"Bound Residues"	37.5	(Includes residues solubilized by 2N HCl reflux (G-19) and 6N HCl reflux (G24))
Organosoluble	8.3	
Water soluble	29.2	(G-21, G-25)
Unextractable	8.6	(After 80% MeOH extraction and 2N, 6N reflux)
Precipitate	13.2	(6.8% was dioxane soluble. Of the remainder, 2.5% was DMSO soluble.)

* Percent recovery was 78.0%.

Clearly, much of the radioactivity remains water soluble. Therefore, an attempt was made to characterize the water soluble metabolites. Straw was extracted with 80% MeOH and subjected to a fractionation scheme (Figure 5 in the petition volume). TLC analysis revealed the presence of 21 ^{14}C-labeled metabolites, each representing from 0.3 - 1.5% of the TRR and collectively accounting for 15.9% of the TRR (Table V in the petition volume). Metabolites in one of the two major metabolite fractions were isolated by HPLC and hydrolyzed with 2N HCl and 6N HCl. Four of 9 HPLC-isolated metabolites could be partially converted to EtOAc-soluble triazinone species (t-BuOH-DADK, 3-amino-DA and DADK). The other 5 were essentially resistant to 2N or 6N HCl hydrolysis.

Association of Metribuzin and Metabolites with Cellulose and Lignin

The nature of the "bound residues" in wheat straw was further examined using two published procedures:

1. Klason Method. (Crawford, D.L., R.L. Crawford and A.L. Pmetto, III. 1977. Preparation of Specifically Labeled ^{14}C-(lignin)- and ^{14}C-(cellulose)-lignocelluloses and Their Decomposition by Microflora of Soil. Appl. Environ. Microbiol. **33**: 1247-1251.) Wheat straw was extracted twice with 80% MeOH. The remaining residue was then extracted with hot water (60-80°C) for 4h. The residue was then Soxhlet-extracted with benzene:ethanol (1:1) for 4h. Finally, the residue was extracted overnight with water at room temperature. In this way 65.7% of the TRR was extracted (Table X in the petition volume). The residue was then stirred in 72% H_2SO_4 for 4h. The extract was diluted five-fold with water and filtered to collect the black, tarry residue (lignin). It was found that 10.3% of the TRR was present in the 72% H_2SO_4 extract and 24.1% was present in the insoluble lignin. The H_2SO_4-soluble fraction consists of proteins, cellulose or other carbohydrates and/or low molecular weight, soluble lignins. Over 95% of the administered radioactivity could be recovered in this way.

2. Langebartels and Harms Method. (Langebartels, C. and H. Harms 1985. Analysis for Non-extractable (Bound) Residues of Pentachlorophenol in Plant Cells Using a Cell Wall Fractionation Procedure. Ecotox. and Environm. Safety **10**: 268-279.) In the second study, ^{14}C-metribuzin-treated straw was subjected to the multiple fractionation scheme given in Figure 4 of the petition. Radioactivity was determined after each extraction step.

After aqueous and organic extractions, the residue was sequentially extracted with α-amylase (to solubilize radioactivity associated with starch), pronase (to solubilize radioactivity associated with protein), and pectinase (to solubilize radioactivity associated with pectin). The residue remaining after the enzymatic hydrolyses was stirred in dioxane:water (9:1) at room temperature for 90h. and filtered. The material remaining was suspended in 100 mL of dioxane:2N HCl (9:1), heated at 70°C for 5h., and filtered. Both the dioxane filtrates contained radioactivity associated with lignin. The material remaining after the lignin was removed was stirred in 24% KOH at room temperature for 24h. and the solution filtered. The filtrate contained radioactivity associated with hemicelluloses. The remaining residue was stirred in 72% H_2SO_4 for 4h., neutralized and filtered. The filtrate contained radioactivity associated with cellulose. The remaining insoluble material was combusted to $^{14}CO_2$.

Results are given in Table 3:

Table 3

Extraction Component	Radioactivity (% of TRR)
Aqueous and Organic Soluble	58.7
Starch	7.9
Protein	2.7
Pectin	2.4
Lignin	26.4
Hemicellulose	1.1
Cellulose	0.6
Insoluble	0.2

The percent recovery of administered radioactivity was 82.1%.

Results from both methods are consistent. We conclude that about 25% of the TRR in metribuzin-treated wheat straw is associated with lignin. Another 10-15% is associated with other biopolymers, such as starch or protein.

The petitioner also conducted two auxiliary hydroponic wheat metabolism studies using ^{14}C-DA and ^{14}C-DADK. The first study established that DA was an intermediate in the metabolism of metribuzin to 3-amino-DA. Since the latter can be obtained by acid hydrolysis of a large number of water soluble metabolites, it is likely that 3-amino-DA is not a true metabolite but represents a portion of a more complex one. The second study, using DADK, produced few water soluble metabolites, implying that these water soluble metabolites are formed from attack at the carbon atom bearing the methylthio group (present in parent or DA but not in DADK). No detectable 3-amino-DA was found, implying that the metabolite (fragment) does not arise from transamination of DADK.

SUMMARY AND COMMENT

From the various wheat studies, we conclude that metribuzin in wheat is metabolized to various triazinone metabolites (DADK, DA, DK) and numerous water-soluble metabolites, most of which could not be converted to organo-soluble species with 2N HCl or 6N HCl. None of the unidentified metabolites was present at more than 3% of the TRR. By 33 days after application, a large percentage of the metribuzin residue had become "bound". Two independent experiments have demonstrated that the bound residue is associated with lignin. That this residue was not released by 72% H_2SO_4 is a good indication that the binding is irreversible.

Characterization of metabolites in wheat grain was not so extensive as in straw, probably because residue levels were more than five times lower. Unlike straw, grain does not contain a

substantial amount of lignin, so we would not expect to find residues associated with that polymer. We do note that almost 50% of the TRR consisted of water-soluble residue that apparently was not characterized (denoted G-11, G-21, G-25 in Figure 4). While we do not expect the (unbound) metabolic profile to be qualitatively different from that in straw, the petitioner should characterize the metabolites in the aqueous phase using TLC or HPLC. Any individual metabolite present at over 10% of the TRR should be further characterized using techniques such as NMR and mass spectrometry.

We note that results of the previously submitted alfalfa metabolism study, discussed above, are qualitatively similar to those from the wheat grain study in that more than one-half of the residue was found to be water-soluble.

We conclude that the nature of the residue in wheat forage and wheat straw is adequately understood. The residue to be regulated consists of parent and its triazinone metabolites. Additional work is necessary on wheat grain.

Comparison of Metabolism Study Extraction Procedures With Procedure of Residue Analytical Method

Before leaving plant metabolism we must ask the question: Will the proposed analytical method adequately determine the triazinone metabolites? As described below, metabolites are released in the residue analytical method by refluxing the sample for 1 h. in 4:1 acetonitrile:water. (See PP#6F1783/FAP#H5133, Report No. 40901.) This reportedly will release triazinone metabolites in concentrations equivalent to those released by 2N HCl relux (of unspecified time). Examination of the extraction procedures used in the two metabolism studies suggests that the releasable triazinone metabolite residue in wheat grain and forage will be adequately determined by the analytical method but that the corresponding residue from wheat straw and soybeans would not be.

Of the identified triazinone metabolites and parent extracted from wheat grain, none was released by conditions more severe than 2N HCl reflux, although reflux times were as long as 4 hours. From forage, DADK amounting to 2.2% of the TRR was released by an additional 6N HCl reflux, but this amounted to less than 4% of the released residue and this percentage is undoubtedly lower than the precision of the residue analytical method. However over 25% of the metribuzin residue was released from wheat straw by 6N HCl or 1N NaOH reflux. Similarly, over 31% of the triazinone metabolites released from soybean tissue resulted from glucosidase hydrolysis or 6N HCl reflux. The corresponding percentage from soybean seeds was 20.4% (glucosidase hydrolysis only).

Because of our doubts concerning the adequacy of the analytical method, the registrant should analyze the wheat straw and soybean tissue from plants treated with radiolabeled metribuzin using the standard residue analytical method and compare the results to those from the metabolism studies.

Animal Metabolism

The Registration Standard concluded that the nature of the residue in ruminants and poultry was not adequately understood.

Six radiolabeled studies were reviewed in the Standard. In the first study, two five-year old lactating goats received [5-^{14}C]metribuzin daily for 8 days at either 0.02 ppm or 0.2 ppm in the diet. Goats were sacrificed 6.5 hours after the last dose. At the 0.2 ppm dose level, maximum total ^{14}C residues in milk were 0.002 ppm at six days. Total ^{14}C residues were 0.056 ppm in liver and 0.014 ppm in kidney from the goat dosed at 0.2 ppm and 0.019 ppm in liver and 0.006 ppm in kidney from the goat dosed at 0.02 ppm. No ^{14}C residues were detectable in the remaining organs (various muscle and fat tissues) studied. Residues were not characterized.

In a second study, a lactating dairy cow received daily oral doses of unlabeled metribuzin at 0.2 mg/kg body weight for 7 days followed by a single oral dose of [3-^{14}C]metribuzin at a dose level equivalent to 7 ppm in the diet. The animal was sacrificed after 8h. Radioactive residues in milk peaked at 0.05 ppm at 2h. The residue consisted of metribuzin (44%), DA (12%), DK (15%) and DADK (2%) -- 73% of the TRR could be identified. Residues in tissues were 0.02 ppm (muscle), 0.04 ppm (heart), 0.17 ppm (kidney), 0.17 ppm (fat) and 0.56 ppm (liver). Percent characterization varied from 96% in fat (parent constituted 90% of the TRR) to 32% in liver and 22% in kidney. Characterization was by TLC, but no data were submitted.

The Registration Standard concluded that the residue had been inadequately characterized and objected to the dosing method -- unlabeled metribuzin for 7 days followed by a single dose of radiolabeled compound.

In another study (PP#5F1628, Report # 40768) two male pigs received a single oral dose of [5-^{14}C]metribuzin at 1.74 mg/kg, equivalent to about 20 ppm in the diet (assuming an intake of 1250 g-feed/day). Residue levels in tissue ranged from 0.3 ppm in brain to 5.0 ppm in liver. Tissues were extracted with methanol, and the remaining solids were autoclaved for 90 min. at 121°C in 1N HCl. All of the ^{14}C-activity in fat was organosoluble and was identified as parent (85%) and DA (15%). A similar situation prevailed for muscle, heart and brain, but the relative propotions of DA were larger. Although most of the residue in kidney and liver was organosoluble (73% and 84%,

respectively), the residue consisted principally of polar metabolites. These could be hydrolyzed by 1N HCl in an autoclave at 121°C for 90 min. to release the triazinones. Additional triazinones were released through acid hydrolysis of the solids remaining after methanol extraction. The total percent identified radioactivity from each tissue varied from 69% in kidney to 100% in back fat. There is not an explicit metabolite listing for liver or kidney, but the text of Report 40768 implies that the major metabolite in each organ tissue was DADK.

The Registration Standard concluded that the nature of the residue in swine was adequately described, but noted that to obtain conjugates of DADK and DK -- the principal components of liver and kidney -- autoclave hydrolysis in acid was necessary.

The fourth and fifth studies described are poultry metabolism studies. In the first of these, 16 hens (5/dose group and one control) received [5-^{14}C]metribuzen at 0.03 ppm, 0.09 ppm and 0.3 ppm in feed daily for 14 days and were sacrificed on the last day of treatment. No ^{14}C-residues were detectable in eggs or tissues from hens at all dose levels, with the exception of one liver sample which contained total ^{14}C residues of 32 ppb.

In the second of these studies, 21 hens received [5-^{14}C]metribuzin as a single oral dose at 25 mg/kg and were sacrificed 24 hrs. posttreatment. Total ^{14}C-residues varied from 0.20 ppm in dark muscle to 6.16 ppm in liver. Metribuzin and the usual triazinone metabolites were quantitated in tissues; but, inspite of an autoclave acid hydrolysis step, unidentified residues varied from 57% of the TRR in fat to 93% in liver.

Rat and dog metabolism studies were also discussed in the Standard. About 60% of the radioactive residue in liver tissue obtained 28 hours posttreatment from rats dosed at 100 mg/kg could be identified as metribuzen, DA, DK and DADK. In muscle, 95% of the TRR was identified as these same metabolites. A similar pattern of residue excretion and distribution was observed in dogs orally dosed at 10 mg/kg. Tissues were examined 24h. after dosing. Acid autoclave hydrolysis released most of the ^{14}C-residues in liver as DADK.

In view of the above studies, the Registration Standard required the following:

> Metribuzin studies utilizing ruminants. Animals must be dosed for 3 days with ring-labeled [^{14}C] metribuzin at a level sufficient to make residue identification possible. Milk must be collected twice daily during the dosing period. Animals must be sacrificed within 24 hours of the final dose. The distribution and characterization of

residues (free and conjugated) must be determined in milk, liver, kidney, muscle and fat.

Metabolism studies utilizing poultry. Hens must be dosed with ring-labeled [^{14}C]metribuzin for 3 days at a level sufficient to permit residue identification. Eggs must be collected twice daily during the dosing period. Animals must be sacrificed within 24 hours of the final dose and residues characterized in eggs, muscle, liver, kidney and fat.

The Standard also required that tissues of ^{14}C-dosed animals be analyzed by enforcement methodology to verify that conjugated metabolites of concern are determined by the method.

In response to the Registration Standard, two studies were submitted:

"Distribution and Metabolism of ®SENCOR in a Lactating Goat," R.J. Christopher and S.M. Muller, 12/30/86. Laboratory Project ID SE-4-G. (MRID # 400425-01)

"Distribution and Metabolism of ®SENCOR in Laying Hens," R.J. Christopher and S.M. Muller, 12/30/86. Laboratory Project ID SE-4-P. (MRID # 400425-02)

Ruminants

Metribuzin (SENCOR-5-^{14}C) labeled in the carbonyl position and having specific activity of 20.8 mCi/mmole was synthesized with purity of 99.5%, as determined by TLC.

Two lactating goats were used in the study. One served as a "comparison" (not a control, for it was apparently not sacrificed). The other, weighing 57 kg, was administered 3 doses consecutively at intervals of 24 hrs. of radiolabeled metribuzin diluted with nonradiolabeled metribuzin to a specific activity of 3.02 mCi/mmole. The treatment rate was 10 mg/kg of body weight, and the dose was administered in capsule form via balling gun. Assuming that a 57 kg goat consumes about 3 lbs. dry food/day, the concentration in the diet would be about 410 ppm (≈70x the maximum expected level in animal feeds). Urine and feces were collected daily and stored frozen until analysis. Milk samples were collected twice daily, and the last sample taken immediately before sacrifice. Milk was stored at -10°C until analysis. The goat was sacrificed 4 hrs. after receiving the final dose.

Extraction Procedures

Liver, Kidney, Muscle. The extraction schemes are very similar and are shown in Figure 5.

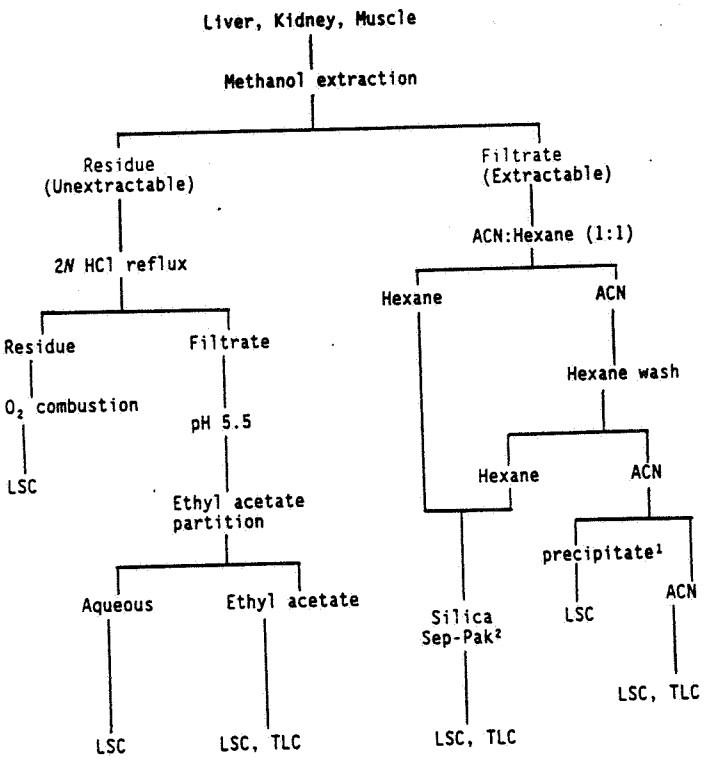


Figure 5. Extraction scheme for goat liver, kidney and muscle samples. (Note: ACN is used here as an abbreviation for acetonitrile.)

[1]Observed only with extracts of muscle.

[2]Additional cleanup procedures of the hexane fraction from methanol extracts of muscle were not performed.

Samples were extracted with methanol and filtered. The filtrates were rotary-evaporated to a volume of approximately 50 mL, then partitioned with 150 mL acetonitrile:hexane. The fractions were separated, and the acetonitrile fractions partitioned once more with hexane. The hexane fractions were combined. Acetonitrile fractions from liver and kidney were concentrated to 1 mL and analyzed by one- and two-dimensional TLC. When the acetonitrile fraction from muscle was concentrated, a water-soluble precipitate formed which contained less than one percent of the sample radioactivity. The precipitate was discarded, and the concentrated acetonitrile

fraction was analyzed by one-dimensional TLC. Radiocarbon levels in the hexane fractions from muscle were about 2.5% of the total and were not studied further. However, substantial fractions of the liver (10.6%) and kidney (13.8%) methanol-extractable radioactivity partitioned into hexane. The hexane fractions from these tissues were purified through silica gel Sep-Pak cartridges using 75% methanol as eluent. The eluates, which contained 75-80% of the radiocarbon present in the hexane fractions, were concentrated and then analyzed by TLC.

The extracted solids from liver, kidney and muscle were refluxed in 2N HCl at 100°C for 1.5 hrs. and filtered. After neutralization, the filtrates were partitioned with ethyl acetate, and aliquots of the ethyl acetate and aqueous fractions were analyzed for radiocarbon by LSC. The ethyl acetate fractions from each tissue were concentrated to about 1.0 mL and analyzed by TLC. Radiocarbon remaining with the solids was quantitated by combustion to $^{14}CO_2$ followed by LSC.

Fat. Almost all the radiocarbon was extracted by methanol. Hence, the remaining residue was not subjected to acid reflux but was combusted to $^{14}CO_2$ and analyzed by LSC. The filtered methanol extract was partitioned with acetonitrile:hexane. Little radioactivity was found in the hexane fraction. The acetonitrile fraction was concentrated and analyzed by TLC.

Milk. Milk was extracted with acetonitrile. The remaining milk solids were extracted with methanol. The acetonitrile and methanol fractions were combined, concentrated and partitioned with hexane. Hexane contained only low levels (1.3%) of radiocarbon and was not analyzed further. The acetonitrile:methanol fractions were further concentrated to a volume of 1 mL. The precipitate that formed was found to have low levels of radiocarbon (≈3%) and was discarded. The acetonitrile:methanol fractions were subsequently analyzed by TLC. Radiocarbon associated with the milk solids after extraction was determined by combustion to $^{14}CO_2$ followed by LSC.

Results

Urine and fecal samples were analyzed for total radioactivity. Over a three day period 60% of the administered radiocarbon had been excreted.

Tissue and milk analyses are shown in Table 4.

Table 4

Sample	Parts per Million (PPM) Metribuzin-Equivalents
Liver	12.66

Kidney	4.27
Fat	0.97
Muscle	0.44
Milk, Day 1, PM	1.32
Day 2, AM	0.25
Day 2, PM	1.76
Day 3, AM	0.25
Day 3, PM	2.09

As expected, highest residues were found in liver and kidney. Analysis of milk samples collected within 8 hrs. after receiving the daily treatments -- i.e., the PM samples -- were more than 5x higher than the samples collected 24 hrs. after dosing -- the AM samples. This suggests a rapid transfer of metribuzin residues into milk from the gastro-intestinal tract.

Almost all of the milk (99.4% of the TRR) and fat (97.5%) metribuzin residue could be extracted into methanol. Only 66.2% of the liver residue could be so extracted, but an additional 28.5% was released on acid reflux. Residue in kidney was 89.1% methanol-extractable, and an additional 8.9% was released on acid reflux. Corresponding percents for muscle were 91.8% and 4.6%. However recoveries based on total residues determined in tissue (Table 4) have not been reported. This information should be submitted.

The complete distribution of metribuzin and its metabolites is given in the following table, which is a composite of Tables IV-VI in the petition.

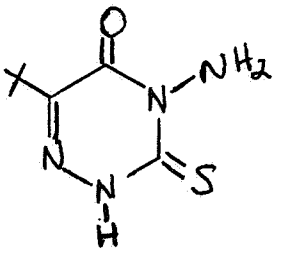
Table 5

	Percent Sample Radiocarbon				
	Liver	Kidney	Muscle	Fat	Milk
Methanol-Extractable Fraction					
1. ACN-Soluble Fraction					
Metribuzin	7.7	2.7	23.5	44.9	2.3
Butylthion	4.1	3.7	19.6	23.7	--
2-Methyl-DADK	0.5	0.9	2.7	2.9	--
DA	6.1	10.6	24.4	5.4	0.6
DADK	0.9	1.5	4.7	2.3	0.5
DK	0.4	1.5	1.3	1.9	0.3
Subtotal	19.7	20.9	76.2	81.1	3.7
Unknown #6	--*	--*	2.3	--	--
Unknown #7	5.2*	7.1*	--	9.2	0.9
Unknown #8a	3.8	19.7*	--	--	43.0
Unknown #8b	--*	18.1*	--	--	40.9
Unknown #9	17.6	1.8*	--	--	--*
Unknown #10	--	4.7	--	--	5.9
Diffuse	9.3	2.9	4.3	2.4	0.7
Origin	<0.1	0.1	5.5	3.1	<0.1
Subtotal	35.9	54.4	12.1	14.7	91.4

2. Hexane-Soluble Fraction						
Unknown #8a		--	2.0	--	--	--
Unknown*#9		1.3	4.5	--	--	--
Diffuse		6.9	3.9	--	--	--
Origin		<0.1	<0.1	--	--	--
(Lost Sample)		2.4	3.4	--	--	--
	Subtotal	10.6	13.8	2.5	1.7	1.3
3. Precipitate		--	--	1.0	--	3.0
Unextractable						
Acid Reflux						
1. Ethyl Acetate Soluble						
DADK		3.9	1.9	0.8	--	0.06
DK		11.5	1.8	1.1	--	--
Unknown*#3		1.8	0.2	--	--	--
Diffuse		2.3	0.5	0.7	--	--
Origin		1.8	0.2	0.3	--	--
	Subtotal	21.3	4.6	2.9	0.0	0.06
2. Aqueous		7.2	4.3	1.7	--	--
Bound		5.3	2.0	3.6	2.5	0.0
	Total	100.0	100.0	100.0	100.0	100.0

* Subjected to acid hydrolysis. Refer to following table (Table 6).
** Refers to sample radiocarbon which did not show discrete bands on TLC plate.

The two most salient features of this table are the very low percentage of identified metabolites in milk and the presence of butylthion in all tissues. This metabolite has not been isolated from metribuzin-treated plants. Its structure is the following:



Unknown #'s 8a and 8b, the principal metabolites in milk, eluted as a single component on the TLC plate (one-dimension). HPLC analysis further resolved this component into two radiolabeled products. Co-chromatographic studies on TLC using 3 different solvent systems and HPLC revealed identical chromatographic behaviour to metabolites identified as 8a and 8b from kidney. However, any effort to establish the chemical identity of these unknowns has not been reported.

Certain of the unknown metabolites -- those denoted by an

asterisk (*) in Table 5 -- were treated with acid in an effort to release the unconjugated species. Exact conditions of treatment are not stated. As an example, unknown metabolites #7, #9 and #10 isolated from kidney (Table 5) were treated with 2N HCl on an individual basis. Metabolites 8a and 8b were treated with 6N HCl. Subsequent extraction of the hydrolysates with ethyl acetate revealed the presence of 4 radiolabeled components, of which DADK comprised 30.6% of the TRR. Metabolites released following acid treatment of liver, kidney and milk are given in the following table:

Table 6
Metabolites Released Following Acid Treatment of Selected Unknown Metabolites

Component	Percent of TRR		
	Liver	Kidney	Milk
DADK	3.2	30.6	0.6
DK	4.5	3.5	--
Unknown #1	4.4	7.6	3.2
Unknown #2	--	0.3	--
Diffuse	2.3	1.7	0.5
Aqueous	12.2	7.7	1.6
Subtotal	26.6	51.4	5.9

Percentages of metribuzin and metabolites isolated and identified if given in the following table, Table 7 (Table VII of the petition.

Table 7
Metribuzin and Metabolites Isolated and Identified in Tissues and Milk

Compound	Percent of TRR				
	Liver	Kidney	Muscle	Fat	Milk
Metribuzin	7.7	2.7	23.5	44.9	2.3
Butylthion	4.1	3.7	19.6	23.7	--
2-Methyl-DADK	0.5	0.9	2.7	2.9	--
DA	6.1	10.6	24.4	5.4	0.6
DADK					
Free	0.9	1.5	4.7	2.3	0.5
Conjugates	7.1	32.5	0.8	--	0.6
DK					
Free	0.4	1.5	1.3	1.9	0.3
Conjugates	16.0	5.3	1.1	--	--
TOTAL	42.8	58.7	78.1	81.1	4.3

Comment

From Table 7 we conclude that the nature of the residue in muscle and fat is adequately understood. Although the percent identified residue in liver is not high, we note that there is only one compound, "Unknown #9" (Table 5), in which an unidentified component was greater than ten percent of the TRR. That component could be partially hydrolyzed in 2N HCl (Table 6), but it is not clear which hydrolysis products can be ascribed to

the particular metabolites listed in that table. In contrast to kidney, it is not clear from the text whether the unknown metabolites were separately hydrolyzed or hydrolyzed as a mixture. Further characterization is necessary. Hydrolysis data on this particular metabolite may sufficiently respond to our concern.

Similar considerations apply to kidney. Here, however, we know that the metabolites "Unknowns #8a and #8b" (corresponding to concentrations 0.84 ppm and 0.77 ppm in metribuzin equivalents) were separately hydrolyzed, but we do not know to which particular components of Table 6. From examination of Table 6 it seems likely that these unknowns are conjugates of DADK, but hydrolysis to DADK was carried out in 6N HCl, which could drastically alter the original structure. Unknowns 8a and 8b also constitute over 80% of the metribuzen residue found in milk. For these reasons we require more complete characterization of these two unknowns. The specific hydrolysis data, including time and temperature, should be submitted. Attempts should be made to identify the unknowns by physical methods such as NMR or mass spectrometry. Absolute identification may not be possible, but we would like to know with more certainty the identity of the metribuzin-related subunit. We reiterate that the administered dose was highly exaggerated, and the actual concentration of individual metabolites in animals will be much lower.

Considering butylthion, although ruminant feeding studies in which tissues were analyzed for this metabolite are not available, we can estimate its concentration in meat products using the total radioactive residue results given in Table 4. The maximum concentration of metribuzin residues in the diet is 5.6 ppm (see section on meat, milk, poultry and eggs), and the metribuzin fed in the metabolism study was >70x this dose. Anticipated butylthion residues derived from this information are shown in Table 8:

Table 8

	Liver	Kidney	Muscle	Fat
Butylthion as % of TRR	4.1%	3.7%	19.6%	23.7%
PPM Butylthion	0.51 ppm	0.16 ppm	0.086 ppm	0.23 ppm
PPM Anticipated Residues of Butylthion (preceding row divided by 70)	0.007 ppm	0.002 ppm	0.001 ppm	0.003 ppm

At these levels we do not consider it necessary that the Metabolism Peer Review Committee formally consider butylthion. DEB does not consider levels such as these to constitute a residue problem. However, TOX should be asked to concur with our opinion.

Representative copies of TLC's used to identify metabolites should be submitted.

The percent recoveries of radiocarbon relative to the radiocarbon determined in tissues by combustion to $^{14}CO_2$ should be reported. Small percentages of lost radioactivity were reported for liver and kidney (Table 5). Does this represent the only unrecovered radiocarbon?

Comparison of Metabolism Study Extraction Procedures With Procedure of Residue Analytical Method

Although there are no residue analyses on animal products in this petition, it is necessary that we know whether the extraction procedure of the residue analytical method will release the same concentrations of metribuzin and its metabolites as did the extraction procedures used in the metabolism studies.

Mobay's residue analytical method is entitled "A Gas Chromatographic Method for Determining Residues of SENCOR and Metabolites in Animal Tissues, Milk and Eggs," (Report No. 42257, PP#6F1783/6H5133). Only the extraction procedure is relevant to this memo. Ground animal tissue (25 g) is extracted for about 16 h. at room temperature with a solution of 25 mL concentrated HCl in 275 mL isopropyl alcohol. Tissues (with the exception of fat) are then heated in the same solution for two hours at 60°C before neutralization and benzene extraction. The residue method was validated by comparison of percent ^{14}C-labeled metabolites released and detected in tissues with corresponding percents detemined in one of the earlier cow metabolism studies. Except for fat, the residue method accounted for more radioactivity extracted than did the actual metabolism study. However, only 28% of the inital radioactivity in kidney could be ascribed to metribuzin or its triazinone metabolites. We have seen (Table 6 and discussion) that a large portion of the kidney triazinone residues were released by hydrolysis in 6N HCl (The exact percentage was not given). Therefore, we cannot say with a reasonable degree of certainty that all releasable triazinone metabolites will be determined by this method. As in the case of plant analyses, the registrant should compare results of the residue analytical method on kidney with corresponding results of the latest metabolism study.

Poultry

Radiolabeled metribuzin (SENCOR-5-^{14}C) was synthesized with a specific activity of 21.9 mCi/mmole and purity 99.2%, as determined by TLC. The radiolabeled product was diluted with unlabeled metribuzen to a specific activity of 2.17 mCi/mmole, and this mixture was used to orally dose five laying hens. The birds were treated with three 25 mg/kg doses administered consecutively at 24 h. intervals. Neither the weight of the

birds nor the average feed weight was reported, but based on data in Morrison's Feeds and Feeding, 1957, p. 970, the dose would be equivalent to a concentration in the daily diet of about 400 ppm. Eggs were collected twice daily, and the contents were separated from shells, mixed and stored frozen until further analysis. Shells were discarded. The hens were killed 1.5 h. after final dosing.

Extraction Procedures

Liver, Kidney. The extraction scheme for liver and kidney is given in Figure 6. Liver samples were extracted with methanol and filtered. The filtrate was rotary evaporated, and the concentrated solution partitioned with acetonitrile:hexane (1:1). The fractions were separated and the acetonitrile fraction partitioned once more with hexane. The hexane fractions were combined, and an aliquot analyzed by LSC. The acetonitrile fraction was further concentrated to a volume of 1 mL. This fraction was then analyzed by TLC using 4 different solvent systems and unlabeled metabolite standards.

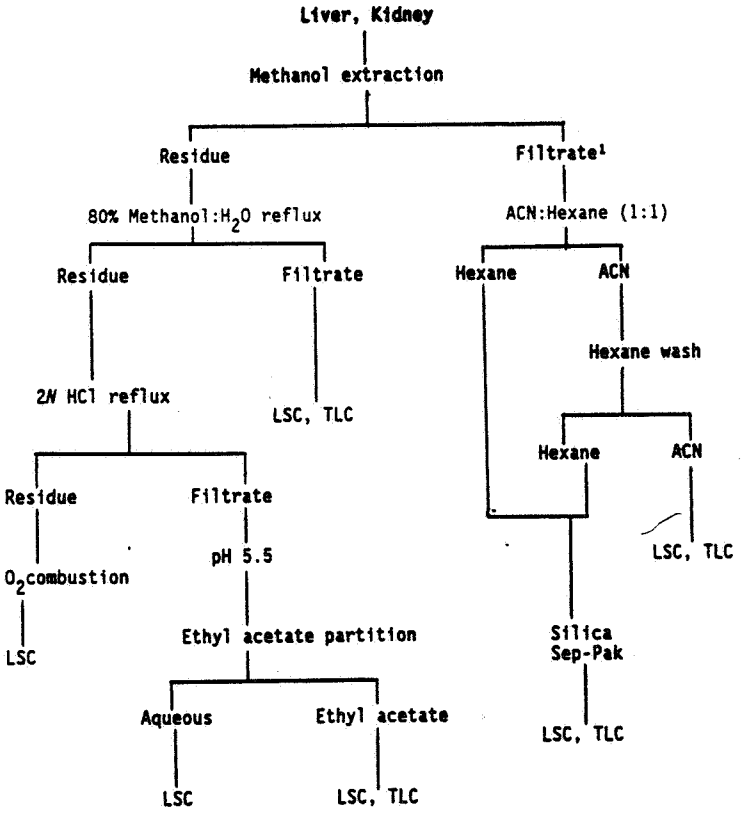


Figure 6 Extraction scheme for chicken liver and kidney samples.

[1]Methanol extracts of composite kidney samples were analyzed directly on TLC and did not undergo further cleanup procedures.

Note: ACN is used here as an abbreviation for acetonitrile.

More than one-quarter of the the methanol-extractable sample radiocarbon partitioned into the hexane fraction. This fraction was applied to a silica Sep-Pak cartridge and eluted with 75% MeOH:H_2O. Using this procedure 80-85% of the radiocarbon present in the hexane fraction could be recovered. The MeOH/H_2O eluates were concentrated and then analyzed by TLC.

The liver solids remaining after the methanol extraction were refluxed in 80% MeOH at 80-95°C for 2 h. The filtrate was concentrated and analyzed by TLC. The remaining filter cake was further refluxed in 2 N HCl for 2 h. and then cooled, neutralized, and partitioned with ethyl acetate. Aliquots of each phase were analyzed by LSC. The ethyl acetate extract was concentrated and analyzed by TLC. Radiocarbon remaining in the extracted solids was determined in the usual way.

Kidney samples were extracted in a similar manner. Due to the low lipid content in the methanol extracts, it was not necessary to perform an acetonitrile:hexane partition. Concentrated methanol extracts were analyzed directly by TLC.

Muscle, Fat, Skin, Gizzard, Heart. The extraction schemne is essentially that for goat liver and kidney (Figure 5). Only the extracted solids from muscle and fat samples were subjected to acid reflux (2N HCl, 2 h., 100°C).

Eggs. Extraction procedures with composite egg samples were limited to those eggs collected on the day of sacrifice (i.e., shell gland eggs). Eggs collected earlier contained much lower metribuzin residues. An egg composite sample was extracted with acetonitrile and the solution partitioned with hexane. The hexane layer contained low levels of radiocarbon and was not analyzed further. The acetonitrile fraction was concentrated and analyzed by TLC. The residue remaining after the acetonitrile extraction was refluxed in 2 N HCl (2 h., 100°C), neutralized and filtered. The filtrate was partitioned with ethyl acetate and both fractions analyzed by LSC. The ethyl acetate fraction was concentrated and analyzed by TLC. The residue remaining after 2N HCl reflux was refluxed in 6N HCl (4 h., 100°C), neutralized, filtered and partitioned with ethyl acetate, as before. The ethyl acetate fraction was cleaned up on a silica Sep-Pak before TLC analysis.

As in previous studies, unknown radiolabeled compounds isolated from tissue extracts following TLC analysis were subjected to acid hydrolysis (2N HCl) for identification of the released radiolabeled metabolites.

Results

Radiocarbon residues in tissues are given in Table 9. As expected, maximum residues were found in liver and kidney.

Table 9

Radiocarbon Residues (PPM) in Tissues and Eggs

Liver	33.6
Kidney	36.3
Muscle	1.6
Gizzard	5.3
Fat	4.0
Skin	4.5
Heart	5.3
Eggs	
Day 1	0.2
Day 2	0.4
Day 3	1.0 (shell gland eggs)

Percent radiocarbon extractable by methanol and 80% methanol reflux varied from 62.0% (liver) to 96.4% (fat). The complete distribution of metribuzin and its metabolites is given in the following table, which is a composite of Tables IV-VIII of the petition volume.

Table 10

	Percent of Total Recovered Radiocarbon							
	Liver	Kidney	Muscle	Fat	Skin	Gizzard	Heart	Egg
Methanol-Extractable ACN-Soluble								
Metribuzin	2.5	1.3	21.5	74.2	52.1	71.7	13.6	14.6
DA	13.0	6.7	51.2	17.2	22.1	7.0	43.0	22.5
DADK	1.9	2.3	1.9	0.6	1.0	0.6	1.5	3.1
DK	--	--	1.7	0.8	0.7	0.6	1.0	1.3
Subtotal	17.4	10.3	76.3	92.8	75.9	79.9	59.1	41.5
Unknown #1	--	--	--	--	--	--	--	2.3
Unknown #2	--*	--	2.1	0.7	--	--	--	--
Unknown #3	1.6*	--*	--	--	--	--	--	--
Unknown #4	1.3*	2.7*	--	--	--	--	--	--
Unknown #5	2.2*	3.1*	--	--	--	--	--	--
Unknown #6	8.0*	3.6*	--	--	--	--	--	--
Unknown #7	--	20.0*	--	--	--	--	--	--
Unknown #8	--	23.4*	--	--	--	--	--	--
Unknown #9	--	2.2*	--	--	--	--	--	--
Unknown #10	--	2.2*	--	--	--	--	--	--
Diffuse	2.4	5.2	<0.1	0.3	2.8	4.3	2.6	--
Origin	2.0	1.3	1.7	1.1	1.7	1.7	3.9	--
Subtotal	17.5	63.7	3.8	2.1	4.5	6.0	6.5	2.3
Hexane Soluble								
Unknown #1a	16.1	--	--	--	--	--	--	--
Unknown #2a	1.8	--	--	--	--	--	--	--
Unknown #3a	2.5	--	--	--	--	--	--	--
Unknown #4a	1.0	--	--	--	--	--	--	--

Origin	0.7	--	--	--	--	--	--	--
Subtotal	22.1	--	2.0	1.5	3.6	4.6	4.1	4.3
Lost Sample	5.0		0.8			0.6		
Acid Reflux Fraction Ethyl Acetate Soluble								
DADK	10.9	15.4	6.3	--	--	--	--	8.9
DK	17.1	3.5	1.6	--	--	--	--	12.8
Unknown #1	1.1	0.4	--	--	--	--	--	--
Diffuse	1.3	0.9	<0.1	--	--	--	--	4.9
Lost	--	--	--	--	--	--	--	0.8
Origin	1.3	1.0	0.5	2.1	--	--	--	2.0
Subtotal	31.7	21.2	8.4	2.1	--	--	--	29.4
Aqueous	3.5	3.1	2.1	1.3	--	--	--	22.5
Unextractable	2.8	1.7	6.6	0.2	16.0	8.9	30.3	--
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

* Subjected to Acid Hydrolysis

From examination of Table 9, we see that the major unidentified metabolites are "Unknown #1a" from the liver fraction and "Unknowns #7 and #8" from the kidney fraction. No attempt was made to identify these compounds, but they were subjected to acid reflux to release triazinone moieties. Unknowns #3-6 and 1a-4a were treated separately as two groups with acid. Similarly, Unknowns #4-10 from kidney were also combined and refluxed with 2N HCl. Released triazinones are shown in Table 11.

Over 20% of the TRR in eggs remained as unidentified water-soluble species after acid hydrolysis (2N, 18.3%, or 6N, 4.2%). It is our opinion that due to the very low residue expected in egg, attempts to characterize this aqueous fraction are not warranted at this time.

Table 11

Compound Released from Acid Hydrolysis of Isolated Poultry Metabolites

Compound	Percent of TRR	
	Liver	Kidney
Methanol-Extractable Unknowns		
ACN-Soluble Fraction		
DADK	3.3	23.0
DK	2.4	3.7
Unknown #1	1.6	7.1
Unknown #2	1.2	10.1
Origin	0.4	2.3
Subtotal	8.9	46.2
Hexane-Soluble Fraction		
Metribuzin	2.3	
DADK	7.2	

DK	3.8
Unknown #2	1.9
Unknown #4	1.3
Origin	1.3
Subtotal	17.8

Not all the radiocarbon activity is accounted for -- in contrast to the goat metabolism study. For example, liver metabolites from Unknowns # 3-6 comprised 13.1% of the TRR. Only 8.9% is accounted for in Table 11. What probably has been omitted is the remaining radioactivity in the aqueous fraction. We conclude that there is no unknown metabolite (or conjugate not containing one of the known triazinone moieties) present at more than 10% of the TRR.

Identified metribuzin residue as percent of the TRR is given in the following table:

Table 12

Identified Metribuzin Residue as Percent of TRR

Tissue	% Identified
Liver	64.4
Kidney	55.9
Muscle	84.2
Fat	92.8
Skin	75.9
Gizzard	79.9
Heart	59.1
Egg	63.2

Comment

From previous tables we observe that the balance of radiocarbon can be attributed to unknowns, origin (very polar), water soluble, or bound residues. No single unknown compound constitutes a major fraction of the residue. Compounds present in significant fractions of the residue are metribuzin, DA, DADK, DK and their conjugates. It is not possible to specify the concentrations to these metabolites relative to each other; for, as the petitioner notes, under acid reflux conditions metribuzin is hydrolyzed to DK and DA is hydrolyzed to DADK. "The presence of DADK and DK in extracts of acid-treated samples, therefore, may be attributed to conjugates of compounds other than DADK and DK and may include SENCOR and DA." The nature of the residue in poultry and eggs is adequately understood pending adequate responses to our questions below.

Just as in the case of the ruminant metabolism studies, the petitioner should submit representative TLC's and percent

recoveries of radiocarbon based on the initial tissue radiocarbon residue, as determined by LSC.

Three metabolism reports were submitted on May 5, 1986 as a response to the Registration Standard and have not as yet been reviewed:

1. "Metribuzin Metabolism in Soybeans. Characterization of the Intraspecific Differential Tolerance," L.N. Falb and A.E. Smith, Jr., J. Agric Food Chem., 1984, **32**, 1425-1428. (Mobay Chemical Company Report No. 88876, EPA Accession # 262890.)

In this study radiolabeled metribuzin uptakes into a susceptible variety, "Semmes", and a tolerant variety, "Coker 338", were compared. After 106 hours treatment by subirrigation, radioactivity in Coker 338 was restricted mostly to the older, more mature plant parts, with little radioactivity moving into the growing points. An autoradiograph of the tolerant cultivar also shows that the radioactivity was largely restricted to the veins. On the contrary, radioactivity in "Semmes" shoots was mostly in the intervenal leaf tissue, and a greater accumulation of radioactivity was noted in the growing points. It was found (by TLC) that metabolism to polar products occurred to a much greater extent in Coker 338. The polar metabolites are unable to penetrate the membranes of the veins and enter into the rest of the leaf, whereas nonpolar parent more readily passes through the membranes.

2. "Alternate Pathways of Metribuzin Metabolism in Soybean: Formation of N-Glucoside and Homogluthione Conjugates," D.S. Frear, H.R. Swanson and E.R. Mansager, Pesticide Biochemistry and Physiology, **23**, 56-65 (1985). (Mobay Chemical Report No. 88943.)

Excised leaves from five to eight week old TRACY M plants were used for metabolite isolation and identification studies. Fully expanded trifoliate leaves were pulse-treated via the cut petiole with either ^{14}C-metribuzin or ^{14}C-DK. Both species were absorbed within 2-3 h. Distilled water was added, and the treated leaves were maintained at room temperature under cool white fluorescent lights for 48 h.

Under these conditions, almost 85% of the metribuzin residue was parent. However, two polar metabolites were identified. Metabolite I (6-7% of the absorbed ^{14}C) was identified as the peptide conjugate of metribuzin -- 4-amino-6-tert-butyl-3-S-(gamma-glutamyl-cysteinyl-ß-alanine)-1,2-4-triazin-5(4H)-one. (The peptide is connected through the sulfur atom of metribuzin.) Identification was based on an analysis of the acid hydrolysis products, CI/MS analysis of the methylated and acetylated derivatives, and FAB/MS (fast atom bombardment mass spectrometry)

analysis of the isolated metabolite. Three other metabolites were isolated. The major one, Metabolite I (percentage of absorbed ^{14}C not reported) was shown to be the malonated N-glucoside of metribuzin. Metabolite II, representing about 0.5% of the TRR, was found to be the N-glucoside of metribuzin, and Metabolite III, less than one percent of the TRR, was tentatively identified as 4-malonylamido-6-tert-butyl-1,2,4-triazin-3,5(2H,4H)dione (N-malonyl-DK).

Comment. The study reveals the metabolic pathway occuring shortly after metribuzin is applied to soybean plants. However the relevance to residue chemistry issues is less clear, for we know that at longer time periods more extensive metabolism occurs.

3. "Absorption, Translocation, and Metabolism of Metribuzin by Downy Brome and Winter Wheat," D.L. Devlin, L.A. Morrow, and D.R. Gealy, USDA-ARS progress report, undated. (Mobay Chemical Company Report No. 88944.)

In this brief report it was shown that the weed downy brome absorbs metribuzin more rapidly than does wheat. Other information is conflicting. One sentence states that downy brome translocated less metribuzin to the shoots than did winter wheat. A sentence on the following page states the opposite. No data are given. DA was reportedly the major metabolite, but the time after application is not given. The report is not useful.

Analytical Method

The analytical method used for all racs in this petition was previously described in Mobay's Report No. 40901, submitted as a part of PP#6F1783/FAP6H5133. The method is entitled "A Gas Chromatographic Method for the Determination of SENCOR and Metabolites in Various Crops", revised 12/11/74. The author is J.S. Thornton. However, the method is only generally described in the present petition. The petitioner should submit all modifications to the original method, however minor these modifications may be, for each of the racs of this petition. These should include GC instrumentation and GC parameters. (We note that sample chromatograms have been included in each separate field trial report.)

According to the original method, the sample to be analyzed is refluxed with 4:1 acetonitrile/water solution to release "bound" residues of metribuzin and its metabolites. The filtered solution is concentrated to remove the acetonitrile, and the aqueous phase is extracted with chloroform. The chloroform is extracted with 0.1 N NaOH, which removes the metribuzin metabolites. The NaOH extract is neutralized and extracted with chloroform. Decyl alcohol (0.5 mL) is added to the chloroform,

and the chloroform evaporated. The chloroform solution which had been extracted with NaOH and should contain metribuzin only is purified on a Florisil column. The eluate is evaporated, and the residue dissolved in benzene for GC analysis. The flask containing the metabolite residues is dissolved in benzene-acetonitrile (90:10) and purified on a silica gel column prior to analysis by GC.

Percent recoveries for each commodity are given in the following table. Commodities were fortified with metribuzin, DADK, DA, and DK

Table 13

Commodity	Spiking Levels (PPM)	Percent Recoveries
Alfalfa Seed	0.01, 0.02, 0.05	50 - 110*
Alfalfa Threshing Chaff	0.01, 0.02, 0.05	65 - 92
Wheat Forage	0.01, 0.02, 0.05	63 - 110
Wheat Forage Hay	0.01, 0.02, 0.05	60 - 100
Wheat Grain	0.05	74 - 94
Dried Peas	0.01, 0.02, 0.05	60 - 110
Dried Pea Hay	0.01, 0.02, 0.05	60 - 100
Corn, Grain	0.01, 0.02, 0.05	60 - 100
Corn, Fodder	0.01, 0.02, 0.05	60 - 110
Asparagus	0.01, 0.02, 0.05	70 - 120

* Two recoveries at the 0.01 ppm level were below 70% (DK at 50%, DA at 60%. In each case a companion recovery was 80%.)

The method is a variant of Method II in PAM II entitled "A Gas Chromatographic Method for the Determination of Residues of SENCOR and Its Metabolites in Potatoes". The initial steam treatment has been replaced with the acetonitrile-water reflux. Report 40901 notes that the one-hour reflux with acetonitrile-water on ^{14}C-metribuzin-treated alfalfa (PHI 49 days) was just as effective in solubilizing activity as repeated reflux extractions or even acid hydrolysis using 2N HCl. However, refer to our comments on the adequacy of this method in the Nature of the Residue section of this memo.

The Registration Standard concluded that this method was acceptable for data collection and enforcement of tolerances.

Animal products were not analyzed in this petition. The Registration Standard concluded that the available method, Report No. 42257, for analysis of metribuzin and its triazinone metabolites in animal tissues, eggs and milk, was adequate for data collection and tolerance enforcement. However, refer to our comments on the adequacy of this method in the Nature of the Residue section of this memo. If the ruminant metabolite butylthion needs to be regulated, an analytical method must be developed for this metabolite.

According to our most recent listing from FDA (5/26/89),

metribuzin was not recovered by multiresidue method #2 (Protocol B) and was completely recovered by Method #4 (Protocol D). Mobay should complete testing through the remaining protocols of PAM I using the Decision Tree for MRM Testing as a guide.

Residue Data

Storage Stability

The Registration Standard, Residue Chemistry Chapter, concluded the following:

> The available data indicate that residues of metribuzin per se and its triazinone metabolites (DK, DA and DADK) are stable (4 months) during frozen storage of animal products and marginally acceptable during frozen storage of plant commodities; however, it should be noted that in carrot samples stored at intervals as short as 4 months, ≈28% (metribuzin per se) and 38% (DK) of the initial residue remained. Therefore we require that any additional data requested in this standard be generated from samples stored (frozen) no longer than 2 weeks prior to analysis.

In fact, frozen stability varies widely with the particular rac. Metribuzin residues in carrots were found to be unstable; but residues in green alfalfa were stable for almost a year, residues in potatoes were relatively stable for about the same time period (DADK had fallen to an average 68.9% of the inital level), and residues in green peas were relatively stable for over two years.

We note that the Registration Standard Guidance Document did not list storage stability as a data gap. There is thus a discrepancy between the Guidance Document and the Residue Chemistry Chapter.

In view of the uncertainties concerning storage stability, the registrant should conduct a storage stability study on field corn grain and forage.

Wheat

A tolerance of 0.75 ppm has been established for combined residues of metribuzin and its triazinone metabolites in/on wheat grain (40 CFR 180.332). Food and feed additive tolerances have been established for the same residues in wheat milled fractions (21 CFR 193.25, 561.41; 40CFR 185.250, 186.250). Tolerances of 2 ppm and 1 ppm have been established for these residues in/on wheat forage and straw, respectively.

The Registration Standard required that wheat grain bearing

detectable weathered residues of metribuzin be processed into germ and milled products. Previous data were obtained from processed commodities derived from fortified wheat grain. The Standard concluded that available data provided adequate support for metribuzin residues in/on wheat grain, forage and straw, but that data were necessary for wheat hay. Field trials were requested in OK, ID and UT, east of the Cascades.

In response to the Standard, Mobay has submitted three reports:

"®SENCOR - Magnitude of Residue on Wheat," W.W. Loeffler, 7/21/87, Laboratory Project ID SE-3052-85/86, Mobay Report No. 94742. (MRID # 402779-05)

"®SENCOR - Magnitude of Residues on Wheat and Wheat Processing Products," J.L. Delk, 9/30/87, Laboratory Project ID SE-3052-85/86, Mobay Report No. 94743. (MRID # 403676-01)

"®SENCOR - Summary of Residue Data on Wheat - Potential for Secondary Residues in Animal Tissues and Products," C.A. Calovich, 9/1/87, Laboratory Project ID's SE-3052-85/86, NBR: 73-155, 74-109, 74-200, Mobay Report No. 94744. (MRID # 403676-04)

Six field trials were carried out in Idaho, Oregon and Kansas. These states accounted for about 28% of the U.S. wheat production in 1982 (Agricultural Statistics, 1985). The choice of states is acceptable. SENCOR DF (75% Dry Flowable Herbicide) or SENCOR 4 Flowable Herbicide was foliar sprayed onto winter wheat by ground or air application. After harvest, samples were stored frozen for up to 317 days before analysis. We note that the inital extraction occurred 2 to 2 1/2 months before injection into the GC. However, spiked controls were similarly analyzed, so we have no questions. Similar analyses were carried out with the other racs. In all cases, the spiking experiments cover the time between extraction and analysis. Results for forage and hay are given in the following table. Only grain from the KS trial was analyzed in conjunction with the processing study.

Table 14a

Metribuzin Residues in Wheat Forage

State	Formulation	Appl. Rate (lb ai/acre)	PHI (days)	Metribuzin Equivalents (ppm) Metribuzin	DADK	DK	DA	TOTAL
Idaho	75 DF	1/2 (ground)	7	1.69	0.04	<0.01	0.05	1.78
			14	0.78	0.03	<0.01	0.03	0.84
	4 F	1/2 (air)	7	0.48	0.05	<0.01	0.07	0.60
			14	1.51	0.02	<0.01	0.02	1.55
Oregon	75 DF	1/2 (air)	7	2.56	0.04	0.01	0.08	2.69
			14	0.61	0.01	<0.01	0.02	0.64

	4 F	1/2 (ground)	7	2.60	0.05	<0.01	0.09	2.74
			14	0.67	0.02	<0.01	0.02	0.71
Kansas	75 DF	3/4 (ground)	8	0.02	0.05	<0.01	0.04	0.11
			14	0.90	0.09	0.02	0.19	1.20
	4 F	3/4 (ground)	8	0.99	0.15	0.03	0.27	1.44
			14	0.53	0.16	0.01	0.11	0.81

Table 14b*

Metribuzin Residues in Wheat Hay

State	Formulation	Appl. Rate (lb ai/acre)	PHI (days)	Metribuzin Equivalents (PPM) Metribuzin	DADK	DK	DA	TOTAL
Idaho	75 DF	1/2 (ground)	7	6.03	0.17	0.09	0.27	6.56
			14	1.42	0.09	0.03	0.09	1.63
	4 F	1/2 (air)	7	5.76	0.15	0.08	0.31	6.30
			14	1.52	0.08	0.02	0.10	1.72
Oregon	75 DF	1/2 (air)	7	8.89	0.13	0.05	0.36	9.43
			14	1.40	0.05	0.03	0.08	1.56
	4 F	1/2 (ground)	7	7.29	0.11	0.06	0.04	7.50
			14	0.05	0.27	0.16	0.39	0.87
Kansas	75 DF	3/4 (ground)	11	0.88	0.17	<0.01	0.15	1.20
			14	0.15	0.22	<0.01	0.23	0.60
	4 F	3/4 (ground)	11	0.55	0.16	0.03	0.17	0.91
			21	0.20	0.16	0.02	0.18	0.56

* Forage samples from ID and OR were field dried two weeks to make hay; forage samples from KS were field dried 5-6 days for hay samples.

The data for wheat forage support the current tolerance of 2.0 ppm (PHI 14 days).

The data for hay would also support a tolerance of 2.0 ppm. However Mobay has proposed a tolerance of 7.0 ppm to conform with previously established tolerances of 7.0 ppm for alfalfa hay and grass hay. Comparable data are available on grass hay, alfalfa hay and sainfoin hay. The maximum use level for each of these racs is 1 lb ai/A -- 2x the maximum single use level for wheat. Only in grass hay did field trial residues exceed 2.0 ppm. Nevertheless, the proposed tolerance is appropriate for reasons of consistancy with previously established tolerances on alfalfa hay and grass hay.

Processing Study

Field trials were conducted in Stilwell, Kansas to obtain grain for processing. Two foliar spray applications of SENCOR 4 Formulation were made to winter wheat using ground equipment. The first was at a rate of 8 oz. ai/A; the last application was at a rate of 24 oz. ai/A. The interval between applications was 45 days. Samples were collected at mature harvest 46 days after the last application.

The residue level of metribuzin in grain was below the detection limit (0.05 ppm). Since our Residue Chemistry Guidelines state that the samples must contain field-treated detectable residues, preferably at or near the tolerance (0.75 ppm), the wheat processing test could not be completed. A new processing study will be conducted at a later date.

Alfalfa

The Registration Standard required residue data from mature alfalfa seed harvested 28 days after the second of two applications of 1 lb ai/A. The applications were to represent fall and spring dormant applications no more than 5-6 months apart. Tests were to be conducted in CA, SD, WI and PA.

In response, Mobay has submitted the following report: "®SENCOR - Magnitude of Residue on Seed Alfalfa", W.W. Loeffler, 7/17/87, Laboratory Project ID # SE-3222-86 (Morse Labs., Inc., CA). Mobay Report # 94745. (MRID # 40779-02)

Field trials were carried out in CO, ID and KS. These states represent 20% of the commercial area, according to the petitioner. Three experiments were conducted in which metribuzin was applied as a dormant foliar spray at the rate of 1 lb ai/A. Both 4 F and 75 DF formulations were used in the tests. Samples of dry alfalfa seed were collected at 183, 231 and 261 days after spray application. Residues of metribuzin and metabolites were <0.01 ppm in all samples. On this basis, Mobay proposes a tolerance of 0.1 ppm.

In the same three trials alfalfa chaff was analyzed. Total metribuzin levels ranged from <0.01 ppm (PHI 261 days) to 0.11 ppm (PHI 183 days). Mobay proposes a tolerance of 1.0 ppm -- the same as that for cereal grain straw. The PHI on the label would be 180 days.

Comment

Because the PHI on the use label for alfalfa seed will be 180 days, the proposed tolerance is acceptable.

Analysis occurred 4-8 months after harvest. There is no discussion of post-harvest treatment of samples. Assuming frozen storage, available storage stability data support the residue analyses.

Based on the submitted data, a tolerance of 0.5 ppm would be more appropriate than one of 1.0 ppm for alfalfa chaff.

Dry Peas

A tolerance of 0.05 ppm has been established for combined

residues of metribuzin and its triazinone metabolites in/on dried peas (40 CFR 180.332). The tolerance was established on the basis of three field trials held in WA. Combined residues of metribuzin and DADK (only) were <0.02 ppm in/on samples harvested 73-90 days following postemergence broadcast application at 0.5-1 lb ai/A.

The Registration Standard concluded that insufficient data were available to assess the established tolerance, and that in the field trials, samples were not collected at or near the established 50 day PHI. The Standard required data on combined residues of metribuzin, DADK, DA and DK in/on dried pea seed harvested 50 days after postemergence application of the 50% WP formulation at 0.38 lb ai/A. Tests should be conducted in the Northwest.

In response, Mobay has submitted the following report:

"®SENCOR - Magnitude of Residue on Dry Peas," W.W. Loeffler, 7/20/87, Laboratory Project ID # SE-1523-86 (Morse Labs, CA), Mobay Report # 94735. (MRID # 402779-03)

Field trials were carried out in Idaho and Washington. SENCOR 75 DF was foliarly applied at the rate of 6 oz. ai/A. Samples of peas and forage were collected 43 and 48 days following treatment. Samples of peas and vines were further dried for 26 and 23 days to obtain the dried pea and vine samples.

Samples were held in frozen storage for a maximum of 303 days before analysis. We consider the storage stability data on green peas sufficient to support dry pea storage. Residue levels in dry peas were found to be <0.01 ppm. Residue levels in dry hay were found to be above the tolerance of 0.05 ppm -- 0.13 and 0.41 ppm. Mobay requests that the tolerance for hay be increased from 0.05 to 4.0 ppm, the current tolerance for soybean hay.

Comment

The data support the current tolerance of 0.05 ppm for metribuzin residues in/on dried peas.

The Registration Standard did not explicitly request data for pea hay. (It noted that the "available data for residues in or on dry pea vines are insufficient to assess the established tolerance..." but did not list this as a data gap.)

Previous data consisted of three dry pea vine samples harvested in WA 73-90 days posttreatment. Residues of metribuzin and DADK (only) were not detected (<0.02 ppm for sum of these species).

Regarding the 4.0 ppm proposed tolerance, results from 37 field trials held in 11 states and Canada on barley straw showed a maximum residue level of 0.81 ppm for metribuzin and its triazinone metabolites. Most residues were nondetectable. Results from 29 trials in 13 states and Canada on wheat straw showed a maximum residue of 0.56 ppm. Additional data on five samples submitted by Monsanto (metribuzin with tank mixes of metribuzin, alachlor and glyphosate) showed a maximum residue level of 0.24 ppm. Higher residues were found in hay from grass (≤8.9 ppm) as well as hay from alfalfa (≤1.86 ppm), but application rates were higher and PHI's were shorter. The proposed tolerance is appropriate because it is consistent with the existing soybean hay tolerance.

In accordance with the Registration Standard, Mobay has changed the commodity definition from "pea vine hay" to "pea straw".

Corn

The Registration Standard concluded that sufficient data were available to support the current tolerance of 0.05 ppm for combined residues of metribuzin and its triazinone metabolites in/on fresh corn. "However, the single residue value presented for a single field corn grain sample is considered too meager to judge the adequacy of the established corn grain tolerance." In other words, almost all the residue data are on sweet corn. The Standard also requested a processing study. Specifically,

> "Residue data for field corn grain harvested at normal maturity (≈70-89% dry matter) after a single preemergence application with the 50% WP or the 4 lb/gal FlC formulation at 0.5 lb ai/A. Tests should be conducted in IA, MN and NE....
>
> "Field corn grain bearing detectable weathered residues of metribuzin must be processed into oil (crude and refined) and milled products....Exaggerated rates may be necessary to obtain detectable residues in or on grain....."

The Standard also stated that submitted data are sufficient to ascertain the adequacy of the corn forage tolerance, but not the corn fodder tolerance because only data from one sample depicting metribuzin residues in/on sweet corn forage (fodder) were submitted. The Standard requested the following:

> "Residue data from corn fodder harvested at normal maturity following a single preemergence broadcast application at 0.25 lb ai/A. Tests must be conducted in representative states.
>
> "Residue data from corn silage harvested from fields treated

with a single preemergence broadcast application at 0.25 lb ai/A."

In response, Mobay has submitted three studies:

"®SENCOR - Magnitude of Residue on Corn," W.W. Loeffler, 7/16/87, Laboratory Project ID # SE-3086-86 (Morse Labs, Inc., CA), Mobay Report # 94739. (MRID # 402779-01)

"®SENCOR - Magnitude of Residues on Corn and Corn Processing Products," J.L. Deld, 9/20/87, Laboratory Project ID # SE-3088-86 (Harris Labs, Inc., NE; Texas A&M, TX). Mobay Report # 94740. (MRID # 403676-05)

"®SENCOR - Summary of Residue Data on Corn - Potential for Secondary Residues in Animal Tissues and Products," C.A. Calovich, 10/1/87, Laboratory Project ID #'s NBR: 74-109, 74-200 (Mobay Corp, MO), SE-3086-86 (Morse Labs, Inc., CA), Mobay Report No. 94741. (MRID # 403717-01)

Residue data from 7 field trials in 7 states are included in the first report. The states were IA, NE and MN, as specified in the Registration Standard, but also CA, IN, NY and WI. These states accounted for 43% of the U.S. corn production in 1982 (Agricultural Statistics, 1985). The 75 DF, 50 WP and 4 F formulations were included in the program. A single preemergence broadcast application at a rate of 4 oz. ai/A was employed. Corn grain and fodder were taken at dry harvest (PHI's 104-166 days).

We note that the application rate is 1/2 of that recommended in the Registration Standard. The label does specify an application rate of 0.25 lb ai/A, but there is no limit on the number of applications.

Discussion of post harvest treatment of the racs prior to analysis in not given. The maximum time period from harvest to analysis (extraction) was 253 days. In view of the apparent differences in storage stability among racs, we cannot unequivocally state that adequate residue data are available for corn. Mobay must produce data showing that metribuzin residues are stable in corn grain and forage for periods up to 253 days. (We note that for this and the other racs of this petition the method validation covers the period from extraction to chromatographic analysis.) Residues of metribuzin, DA, DK and DADK were found to be lower than 0.01 ppm in all cases. Residues in dry corn fodder showed that all metabolites were <0.01 ppm. However, small amounts of parent were found from the MN and one of the NE trials (0.016 and 0.01 ppm, respectively).

The data support the tolerance, but questions about the proposed use (number of applications) and storage stability remain.

Processing Study

Two broadcast sprays using SENCOR 75 DF were ground-applied to field corn in a trial in Kansas. One application was made preemergence at a rate of 8 oz. ai/A and a second was made postemergence at a rate of 24 oz ai/A. The interval between applications was 114 days. Mobay states that the "product label of SENCOR on corn specifies that no more than 2 lbs. of SENCOR DF may be applied per acre in a single crop season with each application being no more than 1 lb". The label we have reviewed, drafted 11/1/87, contins no such restriction, nor, for that matter, any PHI. Mobay should explain this discrepancy. Because of phytotoxicity, further exaggeration of the application rate was considered impossible.

Dry kernels were collected 36 days after the last application. Kernels were frozen immediately after harvest and maintained in frozen storage for 315 days prior to analysis. The only residue found greater than the limit of determination in the whole corn kernels was in the DADK fraction with a 0.03 ppm value. Reanalysis of this fraction on a confirmatory column resulted in the highest residue value of 0.01 ppm. "Therefore, as the confirmatory residue level in the unprocessed commodity was at the limit of determination, analysis of the processed fractions was not undertaken."

Comment

Residue Chemistry Guidelines state that "RAC samples used in processing studies must contain field-treated detectable residues, preferably at or near the proposed tolerance level...". Measurable residues were obtained, even if they were near the limit of determination. It is possible that these would concentrate to levels well above this limit when the grains are processed. Therefore, Mobay should proceed with the processing study.

Corn Silage

No data were submitted for corn silage, but based on the residues observed in/on corn forage Mobay has proposed a tolerance of 0.1 ppm. Levels in silage, which has undergone some fermentation, should be lower than corresponding residues in forage. Because residues in silage will be covered by the forage tolerance, the silage tolerance is unnecessary.

Fresh Corn Cannery Waste

The Registration Standard requested that a feed additive tolerance of 0.1 ppm be proposed to cover metribuzin residues in fresh corn cannery waste. Mobay responds that SENCOR is

registered for use on field corn only, but is proposing the 0.1 ppm tolerance "in the interest of consistency with the registration standard requirement."

We recommend that the tolerance be established. The registrant may register metribuzin for use on sweet corn in the future.

Asparagus

The Registration Standard recommended that Mobay propose a tolerance increase from 0.05 ppm to 0.1 ppm. The recommendation was based on data from 11 field trials held in three states and Ontario, Canada. The tolerance level of 0.05 ppm is exceeded if nondetection levels for metribuzin and metabolites are added. Although not required by the Standard, the registrant has submitted additional field trial data:

> "®SENCOR - Magnitude of Residue on Asparagus," L.G. anderson, 4/15/88, Laboratory Project ID # SE-1855-85 (Morse Laboratories, CA), Mobay Report # 95685. (MRID # 408027-01).

SENCOR 4 F or SENCOR 75 DF was ground-applied in a single broadcast application to mature asparagus at a rate of 32 oz. ai/A, the highest allowable use rate, in two tests conducted in California. Mature spears were harvested 0, 1, 3, 5, and 7 days following application. The PHI as specified on the label is 14 days. After harvest, samples were held in frozen storage between 570 and 617 days until analysis.

Residues from the treated asparagus declined from 3.66 and 2.73 ppm (sum of metribuzin and triazinone metabolites) at PHI 0 days to 0.03 and 0.04 ppm at PHI 7 days. Residues of DK and DA were nondetected at PHI 7 days, so using the Registration Standard's convention, total residues would be reported as "<0.05 ppm and <0.06 ppm". On the bases of these data, earlier data and the Registration Standard recommendation, the registrant has proposed a tolerance of 0.1 ppm.

The data in this report are not supported by sufficient storage stability data. Nevertheless, based on the previously submitted data, the tolerance of 0.1 ppm is appropriate.

Tomato Processed Products

As discussed in the Registration Standard, available tomato processing data indicate that residues may concentrate in catsup and puree processed from metribuzin-treated tomatoes. The Standard recommended that a tolerance of 0.2 ppm be proposed.

In accordance with the Standard, Mobay has proposed a food

additive tolerance of 0.2 ppm for "tomatoes, processed products".

Sugarcane

A tolerance of 0.1 ppm has been established for residues of metribuzin and its triazinone metabolites in or on sugarcane (40 CFR 180.332). The food additive tolerance for sugarcane molasses is listed as 2.0 ppm in 21 CFR 193.25 and 40 CFR 185.250, while the feed additive tolerance is listed as 0.3 ppm in 21 CFR 561.41 and 40 CFR 186.250.

The Registration Standard notes that an increase in the feed additive tolerance for sugarcane molasses from 0.3 to 2.0 ppm was accepted as a result of FAP#5H5151 but this revision was never completed. Mobay has provided a copy of the final rule (43 FR 157:35915, August 24, 1978). Therefore, the feed additive listings in 21 and 40 CFR are both in error. Mobay has proposed a feed additive tolerance of 2.0 ppm in Section F of this petition "only for the purpose of clearing the record on this issue".

The Registration Standard concluded that the available data support the established tolerances in/on sugarcane. however the following additional data were required:

> "Residues must be determined in/on sugarcane forage grown at HI....a pregrazing interval and tolerance for residues must be proposed. Alternatively, a grazing restriction may be proposed.
>
> Residues must be determined in molasses, refined sugar, and bagasse processed from sugarcane bearing measurable weathered residues of metribuzin, DA, DK and DADK...."

Regarding residues in/on sugarcane forage grown in Hawaii, the current label for sugarcane grown in HI carries the restriction: "Do not use treated foliage for feed or forage". Therefore, additional residue data are not necessary.

A processing study (Report No. 35198) was submitted in FAP#6H5151. The study was not criticized in DEB's review (A. Smith, memo of 2/25/77), but the Registration Standard rejected the study because metribuzin, DA and DK were all nondetectable in the representative sample of the rac, so appropriate concentration factors for residues in processed products could not be determined.

In the study in question, samples were taken from a field in Louisiana which had been treated with 3 x 128 oz/A of a 70% WP formulation. This amounts to 2.7 x the label maximum application level of 3 x 3 lb ai/A. The label PHI for LA, TX is 60 days; the PHI in ths study was 176 days.

The residue in the sugarcane sample was found to be 0.09 ppm -- entirely due to the metabolite DADK. The sample was processed, and the fractions were analyzed. The various componenets had the following levels of metribuzin and metabolites: mixed juice (parent - 0.01 ppm, DADK - 0.09 ppm); clarified juice (parent - 0.01 ppm, DADK - 0.10 ppm); sugar (all <0.01 ppm); bagasse (parent - 0.02 ppm, DADK - 0.32 ppm, DK - 0.01 ppm, DA - 0.02 ppm); syrup (parent - 0.05 ppm, DADK - 0.50 ppm, DK - 0.04 ppm, DA - 0.04 ppm); molasses (parent - 0.15 ppm, DADK - 1.43 ppm, DK - 0.10 ppm, DA - 0.10 ppm); mud (parent - 0.02 ppm, DADK - 0.07 ppm, DK - 0.03 ppm, DA - 0.02 ppm). Based on these data, tolerances of 2.0 ppm were proposed for molasses and 0.05 ppm for bagasse.

DEB Comment

Our Residue Chemistry Guidelines stipulate that the residue level in the rac be at or near the tolerance level. Because the tolerance is only 0.10 ppm and there are 4 possible components, it is highly likely that if total metribuzin residues are near the tolerance, one or more of the components will have nondetectable residues. In this case, total residues were certainly "near" the tolerance. We cannot require that residues be equally distributed among metabolites, and since the highest non-DADK concentration did not exceed 0.15 ppm and the metabolites are structurally similar, DEB agrees with Mobay that an additional study is not warranted.

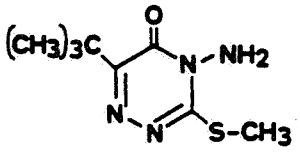
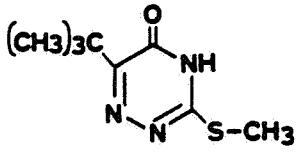
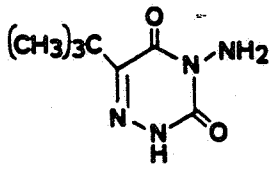
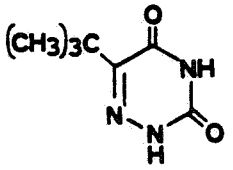
Meat, Milk, Poultry and Eggs.

Tolerances of 0.7 ppm have been established under 40 CFR 180.332 for the fat, meat and meat byproducts of cattle, goats, hogs, horses, poultry and sheep. Tolerances for milk and eggs are 0.05 ppm and 0.01 ppm, respectively.

Adequate ruminant and poultry feeding studies are available and are described in the Registration Standard. Dairy cattle (3/dose group) were dosed with metribuzin for 27-32 days at levels of 3 and 10 ppm in the diet. The maximum total gross residue levels of metribuzin were found in the liver. These values were 0.40 ppm from the 3 ppm feeding level and 1.01 from the 10 ppm feeding level. Maximum levels in milk were 0.004 ppm from the 3 ppm feeding level and 0.006 ppm from the 10 ppm level.

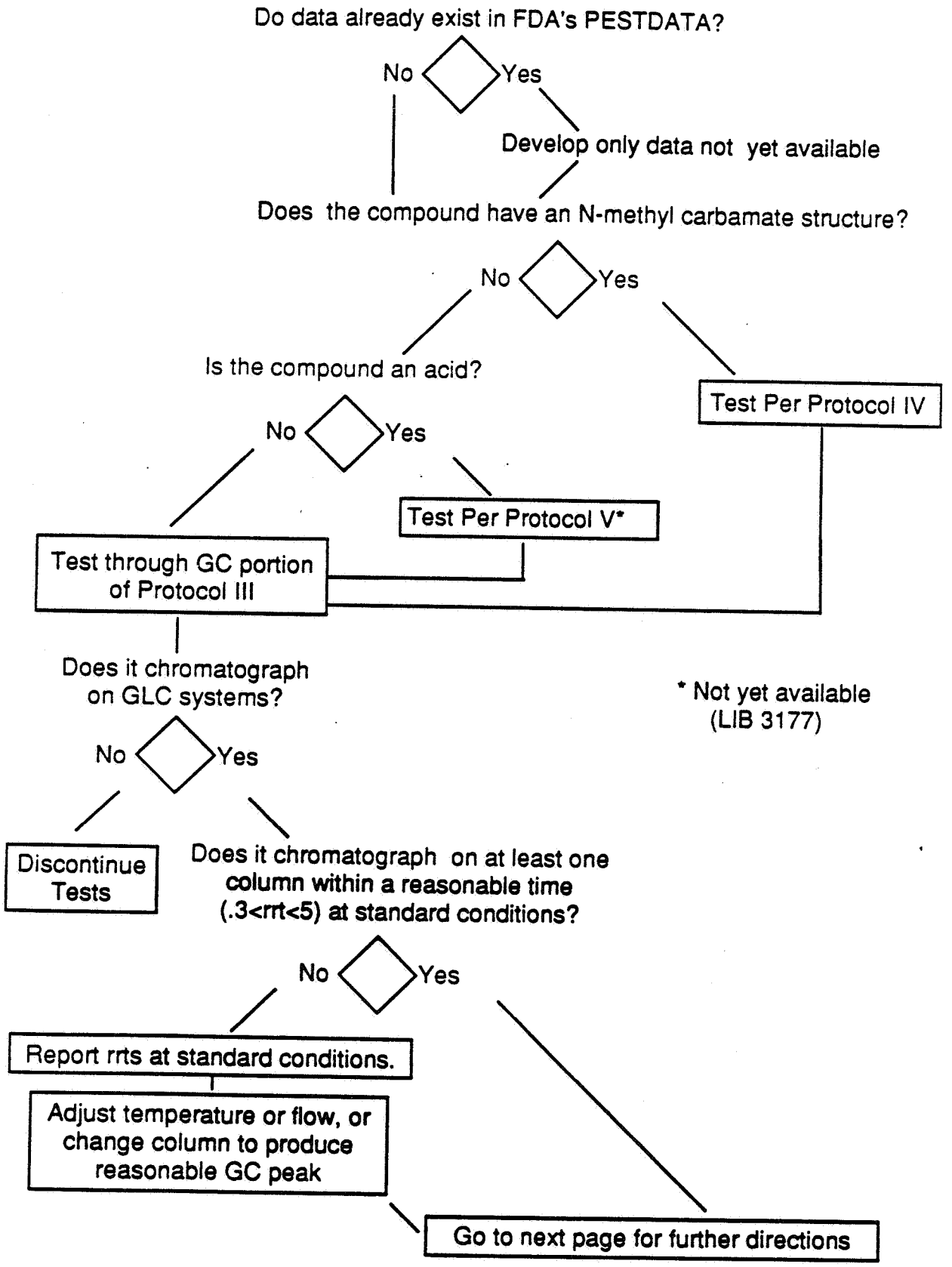
Laying hens (3/dose group) were dosed with metribuzin at 5 ppm, 15 ppm and 50 ppm in the diet for 28 consecutive days. The highest residue levels were found in giblet. Maximum levels were 0.17 ppm from the 5 ppm dose, 0.27 ppm from the 15 ppm dose and 1.80 ppm at the 50 ppm dose. Maximum levels in eggs were 0.020 ppm from the 5 ppm dose and 0.053 ppm from the 15 ppm dose.

Fig. 1. Metribuzin and its metabolites in plants and animals.

CODE	STRUCTURE	CHEMICAL NAME	ABBREVIATIONS
I		4-AMINO-6-(1,1-DIMETHYLETHYL)-3-(METHYLTHIO)-1,2,4-TRIAZIN-5-(4H)-ONE	--
II		6-(1,1-DIMETHYLETHYL)-3-(METHYLTHIO)-1,2,4-TRIAZIN-5-(4H)-ONE	DA
III		4-AMINO-6-(1,1-DIMETHYLETHYL)-3,5-(DIKETO)-1,2,4-TRIAZIN-5-(2H,4H)-DIONE	DK
IV		6-(1,1-DIMETHYLETHYL)-3,5-(DIKETO)-1,2,4-TRIAZIN-5-(2H,4H)-DIONE	DADK

Decision Tree for MRM Testing

DRAFT



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revised May,1988

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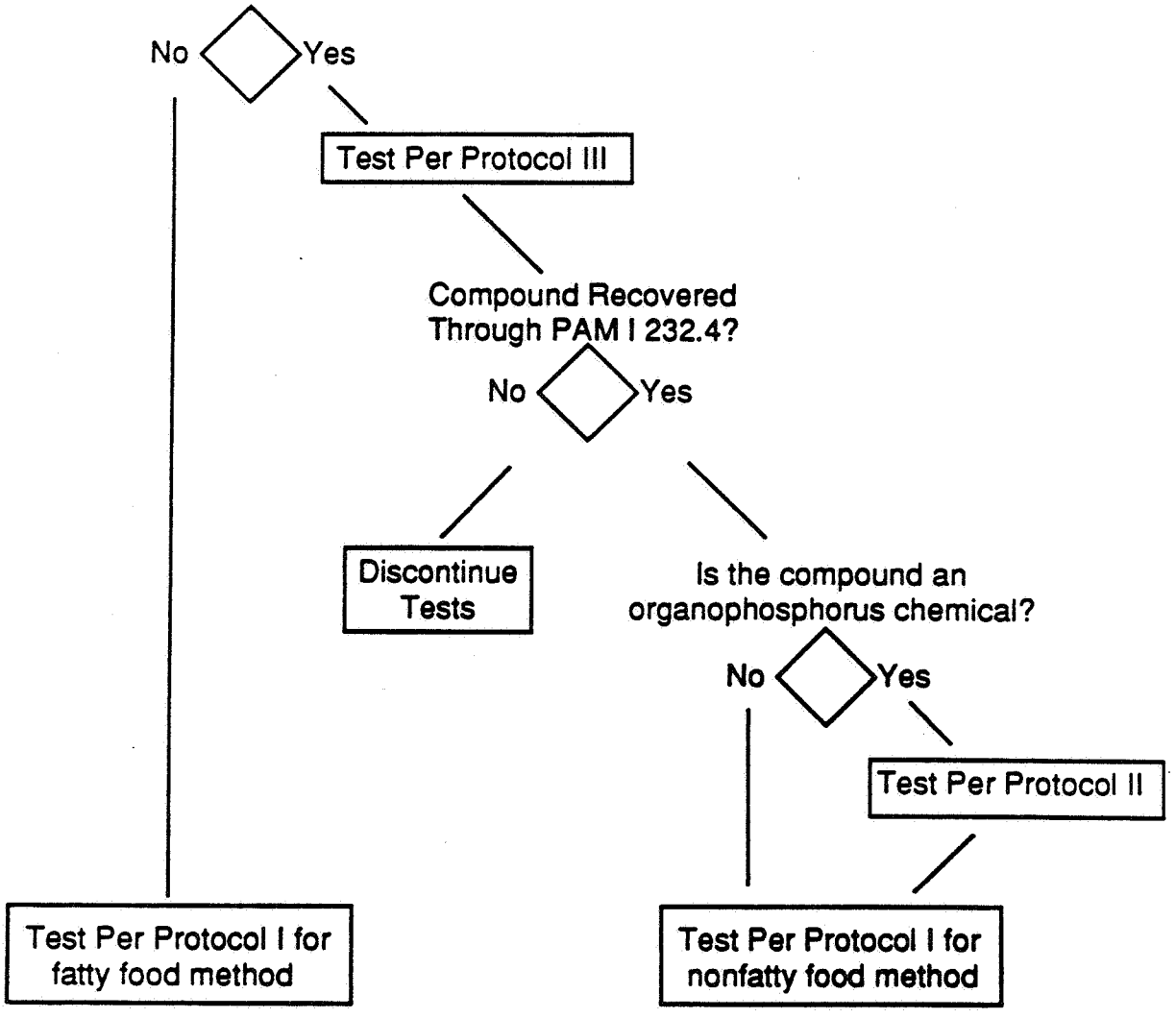
Decision Tree for MRM Testing
page 2

For further study of compounds producing reasonable GC peaks
(Perform recovery studies using adjusted GC conditions if necessary)



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revised May, 1988

INTERNATIONAL RESIDUE LIMIT STATUS

8/17/89

CHEMICAL METRIBUZIN / SENCOR

CODEX NO. ____

CODEX STATUS:

[V] No Codex Proposal Step 6 or above

Residue(if Step 8): ____

Crop(s)	Limit (mg/kg)

PROPOSED U.S. TOLERANCES:

Petition No. 8F 3683 / 8H 5563

RCB Reviewer F100D

Residue: Metribuzin and its triazinone metabolites

Crop(s)	Limit (mg/kg)
alfalfa seed	0.1
alfalfa chaff	1.0
Asparagus	0.1
Barley, forage	2.0
Barley, hay	7.0
Corn, silage	0.1
Corn, fresh, cannery waste	0.1
Pea, Straw	4.0
Wheat, hay	7.0
tomato, Processed Products	0.2
Sugarcane, molasses	2.0

CANADIAN LIMITS:

[ ] No Canadian limit

Residue: parent compound

Crop(s)	Limit (mg/kg) *
alfalfa	0.1
asparagus	0.1
barley	0.1
corn	0.1

MEXICAN LIMITS:

[V] No Mexican limit

Residue: ____

Crop(s)	Limit (mg/kg)

NOTES: Negligible residue type limits